Jameel M. Al-Khayri
Lina M. Alnaddaf
S. Mohan Jain *Editors*

Nanomaterial Interactions with Plant Cellular Mechanisms and Macromolecules and Agricultural Implications

Springer

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Preface

Nanotechnology has the potential to revolutionize agricultural and food production processes. It is imperative to harness the advantages of nanotechnology to enhance plant nutrient availability, improve soil fertility and minimize nutrient losses, develop efficient nanomaterial-packaging contaminants and utilization as a biochemical delivery system in plants. Nanomaterials, owing to their high surface to volume area ratio, are more efficient than most traditional materials. Their nature allows the slow release and promotes efficient nutrient uptake by the crops. Consequently, a reduced number of active chemicals are incorporated in agricultural soils and are absorbed by the plants, thus curtailing the negative impact on the environment. Nanobiotechnology is a promising tool to manipulate plants for the benefit of humankind in terms of sustainable agriculture and food security. Realized agricultural benefits so far are enhanced seed germination, increased plant growth and higher crop yield as well as increased secondary metabolites accumulation for an improved defense system against stress factors including climate change. With increased beneficial prospects and reduced adverse environmental effects, nanomaterials are viewed as instrumental in curbing world hunger.

To fully understand the behavior of nanoparticles in the plant, it is essential to study the mechanism of absorbance and translocation of nanoparticles and their interaction with the plant cellular biochemical compounds and organelles. The movement and accumulation of nanoparticles within the plant are determined by the mode of entry and uptake process as well as biochemical and physical barriers before entering the cytoplasm. Therefore, the current challenge is a systematic understanding of the absorption, translocation, bioaccumulation, barriers and pathways of nanomaterials within the plants. Research concerned with the relationship between nanomaterials and plants has risen dramatically in recent years which have contributed to the current perspectives of nanomaterials influence on cellular processes including photosynthesis, photorespiration and pigment synthesis and accumulation. Other research focused on understanding the impact of nanomaterials on cellular macromolecules including carbohydrates, lipids, nucleic acids, proteins, hormones and antioxidant defense activities. Collectively these processes and biochemical compounds have implications on crop yield. Nonetheless, research is considered in its infancy and more work is needed to fully comprehend the nature, mode of action and utilization of this incredible material in agriculture. The interaction between nanoparticles and plants depends on several aspects such as nanoparticle shape, size, surface characteristics, crystal chemistry and dose. Additionally, it depends on plant genotype, plant age, soil or medium associated plant growth and the application method. Numerous nanoparticles have growth regulating properties causing substantial increases in biomass and even improvement in nutritional quality, whereas, others have toxic effects on plants resulting in morphological, physiochemical and anatomical alterations. This book examines recent progress of the above-mentioned aspects with emphasis on the interaction between nanoparticles and plants on the cellular level. The book starts with an introductory chapter providing an overview of the impact of nanotechnology on plant cell biology and outline the presented topics within the context of the book theme. This is followed by 18 chapters grouped into 3 parts: Part I Cellular mechanisms, Part II Cellular macromolecules and Part III Agricultural implications.

Chapters are contributed by 63 globally recognized scientists from 14 countries and subjected to a rigorous review process to ensure quality presentation and scientific precision. Chapters begin with an introduction providing background and rationale followed by a detailed discussion of the topic accompanied by 71 high-quality color figures and 32 tables. Each chapter concludes with recommendations for future study directions and a detailed list of appropriate sources to encourage further reading. The book is an excellent reference source for plant scientists engaged in research related to cultivation and breeding, biotechnology and nanotechnology. It is suitable for both advanced undergraduate and postgraduate students specialized in biotechnology and agriculture. We extend our appreciation and gratitude to the contributing authors for their assiduous efforts and diligence and to Springer for the opportunity to publish this work.

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Chapter 1
Introduction: Impact of Nanotechnology on Plant Cell Biology

Lina M. Alnaddaf, Jameel M. Al-Khayri, and S. Mohan Jain

Abstract This book focuses on the recent progress of nanotechnology, emphasizing the interaction between nanoparticles (NPs) and plants at the cellular level. Furthermore, it covers understanding of pathways of nanomaterials (NMs) entry into plant cells, their influence on cellular organelle processes, and their influence on total crop yield. It includes 17 chapters, grouped in three sections: (1) Cellular mechanisms, (2) Cellular macromolecules, and (3) Implications of NMs. These chapters provide details on plant response to NMs applications including morphological, physicochemical, and anatomical changes and their effect on plant growth and productivity. The mechanisms of absorbance and translocation of NPs and their interaction with the plant cellular biochemical compounds and organelles are also covered. This book describes the current perspective of NMs' influence on cellular processes including photosynthesis and pigment synthesis and accumulation. Also highlights the current understanding of the impact of NMs on cellular macromolecules, these processes and biochemical compounds have implications for crop yield.

Keywords Adsorption · Cellular macromolecules · Cellular mechanisms · Cell biology · Plant nanotechnology

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1.1 Introduction

Nanotechnology contributes novel tools to impact and enhance crop production and provide an alternative approach for crop improvement. Nanomaterials (NMs) interact with various cellular macromolecules leading to both negative and positive effects, especially the enhancement of plant growth and resistance to different stresses. NMs optimize plant water and nutrient conditions, improving production quality and quantity. On the other hand, NPs are toxic to plant growth and human health via the food chain (Minkina et al. 2020).

The NPs gather in cell walls, tissues, and sub-cellular organelles such as chloroplasts and vacuoles, resulting in decreasing biological activity such as photosynthesis and metabolism in plant cells, reducing the germination rate and decreasing the length of root and shoot. They promote oxidative stress, antioxidant, nutritional imbalance for edible crops and quality of productivity. NPs in plant tissues have an impact on physiological processes (as promote or inhibit) and on the safety of macromolecules and cell organelles (Fedorenko et al. 2020).

There are essential factors related to the NPs and their interaction with plant tissues such as the plant species involved, which have physiological and anatomical differences, chemical characteristics of the plant's cellular surface, also environmental conditions surrounding it (Minkina et al. 2020).

1.2 Cellular Mechanisms

This section describes the interaction of NPs in different parts of plants (roots, shoots and leaves) at the cell organelles level (Fig. 1.1).

1.2.1 Adsorption

The first step is to absorb NPs via the roots and distribute it to plant tissues through modifications such as crystalline dissolution, biomodification and bioaccumulation. Plant roots and plant growth tissues are therefore the primary hosts for receiving NPs (Rao and Shekhawat 2016).

A variety of factors influence the absorption of NMs, including nanomaterial features, plant physiology, application method and environmental conditions.

NMs can interact with microorganisms and soil compounds that can positively or negatively modify absorption efficiency according to the type of NMs. Furthermore, multiple tissues (epidermis, endodermis) and barriers (Casparian strip, cuticle) need to be crossed before reaching vascular tissues, according to the entry point (roots or leaves) (Rajput et al. 2018).

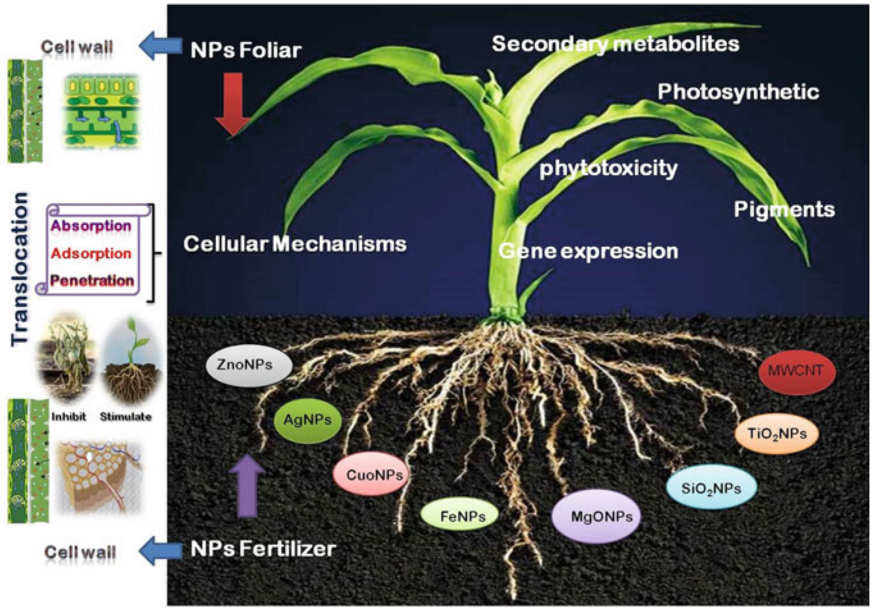

Fig. 1.1 The impact of nanotechnology on plant cellular mechanisms (Constructed by L.M. Alnaddaf)

1.2.2 Penetration

The pathway of NMs to penetrate and uptake inside the cell can be via many ways, such as pore formation: Some NMs may disturb the plasma membrane, causing pore formation to pass via the cell; ion channels: These channels are about 1 nm in size, making it highly unlikely that NMs will penetrate them effectively without significant modifications; carrier proteins: NMs may bind to cell membrane proteins which act as carriers for entry into the cell; endocytosis: The NMs are embedded in the cell as a vesicle which may move in various compartments of the cell; and plasmodesmata (Schwab et al. 2016).

1.2.3 Cell Wall

NPs mainly modify the chemical composition and physical parameters of the cell wall affecting its structure. This modification is related to some factors such as the rate of pectins, structural proteins and phenolic compounds, cellulose, and hemicellulose. Also, pH, cell-wall existing enzymes, and acid cell wall properties regulate its porosity (Milewska-Hendel et al. 2017).

There are some characteristics NPs possessed that allow them to pass through cell walls such as size, shape, dimensions, The surface charge of NPs, composition, concentrations, the amount of fertilizer used, plant species, and conditions of the environment. The interaction between NPs and plants, whether stimulating or inhibiting, varies according to the previous characteristics (Milewska-Hendel et al. 2021).

1.2.4 Translocation

NMs move via plant tissue in the apoplast and the symplast. Also, the importance of the way NMs move inside plants gives indications about where they reach, end and accumulate (Milewska-Hendel et al. 2021).

This book presents several examples of translocation and accumulation of NPs in plant tissues, whether applied as soaked grains, fertilizers and foliar applications. For instance, NMs of carbon-coated iron are transferred to the aerial parts in pea and wheat faster than in sunflower and tomato (Perez-de-Luque 2017).

Also, this process is related to the size of the material and the zeta potential when carbon dots as a model is used in the translocation via the leaves in corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.). Moreover, FeONPs penetrate the roots in (*Cucurbita pepo* L.) and accumulate in the roots' cells without transferring to leaves or flowers due to NPs magnetic properties (Tombuloglu et al. 2020).

1.2.5 Photosynthetic

A nanotechnology is a feasible tool for optimizing photosynthesis. It is related to primary metabolism and is responsible for generating plant biomass. Also induces plant growth and development (Sáez et al. 2017).

The effectiveness of NPs on photosynthetic (enhancing or impeding) and the functionalities of photosynthetic elements vary according to different plant species. This effect appears via an effect on regulatory proteins of the thylakoids, photosynthetic pigment (chlorophyll a and b), the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), carbon dioxide aggregation, adequate grana development and structural stability of mesophyll cells as well as chloroplasts (Kataria et al. 2019).

The enhancing or impeding influences of NPs on photosynthesis and plant growth are varied depending on nonmetallic NPs or metallic NPs. The stimulated reactions of nonmetallic NPs of photosynthesis are generated by biocompatible and reducing the oxidation processes. Also, NPs can be exploited for harvesting more electrons, which in turn, enhancement of plant photosynthesis and increases biomass and crop productivity (Swift et al. 2019).

Conversely, metallic NPs had impeding photosynthesis, which harmfully affects the different photosynthetic mechanisms. Impeding effects can be summed up for

NPs on the photosynthetic as toxicity via production of ROS and reduction each of the following: the net photosynthesis, chlorophyll content, photosystem II activities, the number of thylakoids per grana, the transpiration rate, leaf stomatal conductance, intercellular carbon dioxide concentration rate as well as inhibiting the expression of genes each of photosystem structure and chlorophyll biosynthesis (Poddar et al. 2020). These effects appeared via an application of different types of NPs, such as walled carbon nanotubes (SWCNTs), iron oxide, silver NPs, TiO_2 NPs, CuO NPs, ZnO NPs, carbon dots, Al_2O_3 NPs, Si NPs, Se NPs, Superparamagnetic iron oxide nanoparticles (SPION) for a variety of plant species (Kataria et al. 2019).

Carbon-based NMs interact with accessory pigments in the chloroplasts and chlorophyll-a, and chlorophyll-b and promote the ability of plants to harvest light energy. Whereas, NPs used as artificial antennae permit chloroplasts to absorb wavelengths that aren't essential for photosynthesis. This enhances the ability of plants to interact with light and optimize its capture, which increases the productivity of crops and enables the plants to adapt to different environments, where there is extremely solar radiation or limited resource of light (Aguirre-Becerra et al. 2020).

1.2.6 Pigments

NPs interact with plant pigments (Chlorophyll and derivatives, Carotenoids, Anthocyanins, and Betalains). Once it penetrates the plant chloroplast then the attachment occurs between NMs and pigments plant, which acts as cell protection agents, light-harvesting complexes, transfers absorbed light energy to chlorophyll molecules, dissipates excess energy to the environment and as antioxidant molecules when stress occurs (Nguyen et al. 2021).

Comprehension of the interaction mechanisms of NMs with plant pigments is critical to know its possible side effects on the biochemistry, metabolism, and physiology of plant organisms when NMs are absorbed. The plant produces more than 200,000 different chemical compound types including colored (pigment) compounds (Tripathi et al. 2020).

Engineered NMs interact differently with plant pigments depending on pigment type and their physical-chemical properties resulting in two various basic responses: the first is the pigment content (increase/decrease) by (promoting/inhibiting) the pigment synthesis. The second change is the pigment activities, especially energy dissipation processes and light absorption. Most of the literature indicated that plants respond to different types of NPs by reducing chlorophyll content followed by increasing the accessory pigment's contents. Thus, any functional or structural modifications in plant pigments, especially photosynthetic ones, affect the photosynthetic performance and biomass productivity (Nguyen et al. 2021).

NPs interact with plants to know about the implications of NMs on the plant morphological, physiological, production responses, and biological phenomena, to promote absorption of light wavelengths and optimize its capture and enhance photosynthesis (Santiago et al. 2020).

There are many factors that affect the content of the pigment in plant cells. For instance, biotic and abiotic stress induce the generation of reactive oxygen species (ROS) which increase the expression of genes involved in the pigment biosynthesis pathway, the synthesis route of NMs (green or chemically synthesized) and the usage dose (Tripathi et al. 2020).

1.2.7 Secondary Metabolites

NMs play an essential vital Role in plant cell and tissue culture. NPs have stimulated the biosynthesized secondary metabolites and gene expression for it (Wang et al. 2021).

NPs promote secondary metabolites (for instance, flavonoids, phenolic acids, glucosinolates, terpenoids, and alkaloid compounds). These play an antioxidant activity to enhance plant growth under biotic and abiotic stresses. As well as it decreases drought-induced damage such as *Z. mays* L., improves the quality of fruits under drought stress for *P. granatum* and increases yield. Also, the pharmacological properties of several medicinal plants are imputed to secondary metabolism compounds. Therefore, any change in bioactive compounds by NPs could affect their pharmacological properties and market importance (Ma et al. 2020).

Once the cells absorb the NMs, the metal-base of the NMs is turned into reactive metal ions, which react with functional groups present in a cell leading to a change in their biochemical activity. These interactions are various depending on plant species and tissues, size, solubility, concentration, shape, thermodynamic properties, composition, and surface coating. The main indicators resulting in NMs toxicity in the plant are the reduction of photosynthetic processes and the generation of ROS (Zahedi et al. 2021).

1.2.8 Phytotoxicity

The phytotoxicity caused by NPs is usually triggered by the release of free radicals such as hydrogen peroxide and hydroxyl radical. The excessive production of ROS interacts with various biological molecules and causes different cellular damage. It can also increase the level of oxidative stress, the fragmentation of peptide chains and alter the electric charge. Furthermore, it increases the susceptibility of various proteins to proteolysis, DNA and cell membrane damage, the toxicity of carbon-based NMs, the toxicity of metal and metal oxide NMs and anatomical and morphological changes (Kolenčík et al. 2021).

Exposure to NPs lead to adverse effects on several biochemical and physiological processes in different plant species which affects the growth stages of plants. Also, accumulated NMs in different plant parts can affect human health. In the contrast, some studies have indicated that not all plants treated with NMs demonstrated toxic effects (Tombuloglu et al. 2020).

1.2.9 Gene Expression

In plants, numerous alterations resulting treated with NMs observed in genetic (DNA mutations) and epigenetic (DNA methylation pattern, histone modifications, and RNA interference). They were reflected in many aspects of the plant growth and development, such as cell divisions, chromosome behavior, mitotic aberrations, DNA alterations, and gene regulation related to forming the cell wall, roots, leaves, stress, and water channels (Khan et al. 2019).

SWCNT inhibited histone H3K9 acetylation in Maize seed's response to drought stress. Applying carbon nanotubes (CNT) in *Solanum lycopersicum* L. leads to the downregulation of genes related to roots and leaves. In contrast, increasing the upregulation of genes is related to water channels and stress (LeAqp2). In addition, Multiwalled carbon nanotube (MWCNT) had an impact on the upregulation of marker genes NNtPIP1, NtLRX1, and CycB, related to the formation of cell divisions and the cell wall (Khan 2020).

1.3 Cellular Macromolecules

Carbohydrates, nucleic acid, hormones, proteins, enzymes, and lipids are the main components of plants. These compounds contributed to NPs synthesizing as a safe and eco-friendly method. Also, they are closely associated with stress tolerance, growth and development of plants via increasing or decreasing their percentage when applying several NPs to plants (Khan et al. 2021).

1.3.1 Carbohydrates and Lipids

The chapter on the interaction of NPs with plant macromolecules: carbohydrates and lipids summarizes recent advances in the different effects of NPs to promote or inhibit plant growth, such as seed germination, plant root, and above-ground growth and improve various stress tolerance, which is closely associated with plant carbohydrates and lipids (Shang et al. 2019).

This stimulating or inhibiting is occurring using various types of NPs, for example, silver, selenium, zinc oxide, copper oxide, magnesium oxide, and silicon dioxide in different stages of growth and development of a plant. In addition, this chapter

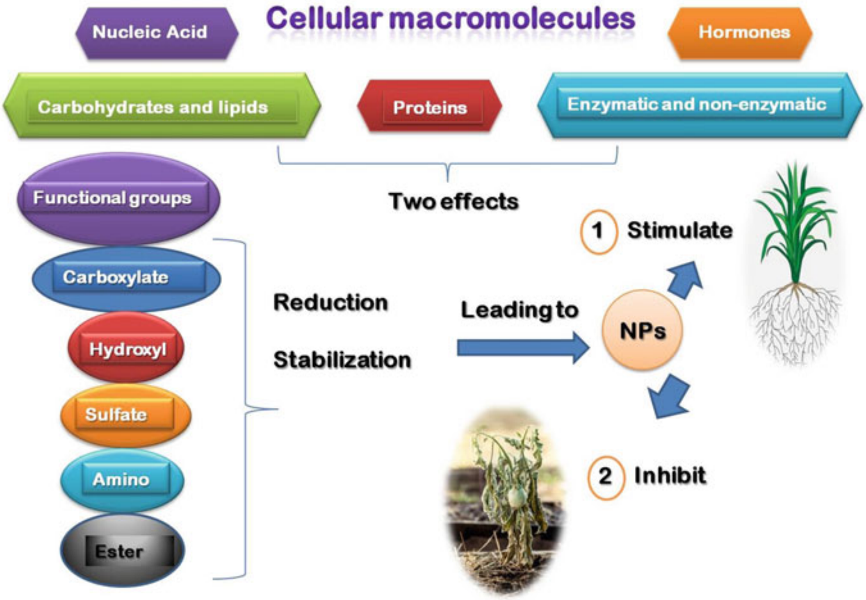

Fig. 1.2 The impact of nanotechnology on cellular macromolecules (Constructed by L.M. Alnaddaf)

discusses the different effects of NPs added as a spray or fertilizer on morphological, biochemical, and productivity indicators of various plants (Khan et al. 2021) (Fig. 1.2).

1.3.2 Nucleic Acid

All genetic information for every living entity exists in nucleic acids (DNA and RNA) (Tan et al. 2009). This book presents a chapter to introduce the NPs interaction with nucleic acids. This interaction relates to type of NPs and concentrations.

In addition, it can use as delivery system DNA and RNA by binding interactions such as carbon nanotubes and bioclay (Hossain et al. 2016). Also, explains in more details the different effects either positive (activate the genes related to stresses) or negative (chromatin condensation and cells shrinkage, damaged DNA, fragmentation of chromosome arms and suppressing transcriptional genes) (Chen et al. 2018).

1.3.3 Hormones

There are five types of hormones that exist naturally in plants: auxin, cytokinin, gibberellins, abscisic acid and ethylene (Gaspar et al. 1996). NPs enhance the various physiological activities of hormones in plants (Weyers and Paterson, 2001). These activities relate to growth (elongation of roots or shoots), maturation, plant tissue culture and biotic or abiotic stress responses in a plant (Yang et al. 2017). While, other side effects are linked to senescence or phytotoxicity (El-Shetehy et al. 2020).

1.3.4 Proteins

Proteins are necessary for all living cells and have several functions such as regulation, cell signaling, catalysis, support, membrane fusion, intra- and intercellular movement of nutrients and other molecules, and structural protection. When NMs penetrate into plant cells, it reacts with carboxyl and sulfhydryl groups and alters protein activity (Anjum et al. 2015).

The NMs reaction depends on the physical and chemical conditions of the cell environment, which is affected by reactive molecules, and temperatures.Also, its sizes and concentrations affect the folding process of a newly synthesized protein (Hossain et al. 2016).

The plant responds to NMs treatment via several indicators such as an increase or decrease in proteins, accumulation or synthesis of new types of proteins that are involved in primary metabolism and production of enzymes that help the metabolic adaptation of the plant. In addition, Increasing proteins -are related to photosynthesis, metabolism, cellular organization, and hormone metabolism (Fig. 1.2) (Hasan et al. 2017).

1.3.5 Enzymatic and Non-Enzymatic Antioxidant

The influence of NMs on enzymatic antioxidant defense activities in plants differs according to the nature and concentration of NPs.

There is a regulatory suitable balance between ROS and antioxidants (enzymatic and non-enzymatic) in natural conditions. However, the plant responds with an increased amount of ROS concentration in conjunction with antioxidants when NPs stress occurs in the plant (Shang et al. 2019).

Antioxidants break down the ROS and scavenge it due to their peculiar structures and detoxify cells, such as oxygen free radicals and lipid peroxidation radicals.

In various researches, the impact of NPs on plant growth was different between promoting and inhibiting (Fig. 1.2) (Khalil et al. 2020).

1.4 Preparation and Features of NM

This book presents the methods to synthesize NM, such as physical, chemical and biological manufacture. Each method has a different feature that is related to its application (Alnaddaf et al. 2021). In addition, emphasizes the advantages of biosynthesis of monometallic NPs and includes some examples of silver, gold, copper, palladium and oxide NPs. Also, explains the factors that affect the NMs traits and behavior which make them able to penetrate plant cells (Shekhawat et al. 2021).

1.4.1 Nanocellulose

Cellulose is a natural polymer derived from agricultural waste and by-products used for the synthesis of various kinds of NMs. This chapter converses the synthesis of nanocellulose. Then, explains in more detail the source, structure, and types of nanocellulose. Also, it highlights the preparation, characterization, and properties of nanocellulose. In addition, it discusses the application of nanocellulose in many sectors (Zhang et al. 2022).

1.4.2 2D-Nanosheets

A chapter presents the 2D-nanosheets based hybrid NMs interaction with the plants. In addition, explains the different methods of synthesis of 2D-nanosheets. Then, emphasizes the interaction of 2D-nanosheets with plants. Moreover, it also highlights to penetration of 2D-nanosheets the seed coats, translocation in the plants and effects on plant growth and development (Lee et al. 2021).

1.5 Implications of Nanomaterials on Crop

The plant has different responses to treatment with NPs, whether morphology physiology and productivity (Hossain et al. 2020) (Fig. 1.3).

This book highlights the mechanism of NPs interaction in seed and various effects on seed germination and root growth, shoots, leaves, flowers and fruits. In addition, this book includes examples of many types of NPs. Also, their role to improve crop productivity by mentioning features of NPs in crop quality and quantity improvement (Rivero-Montejo et al. 2021).

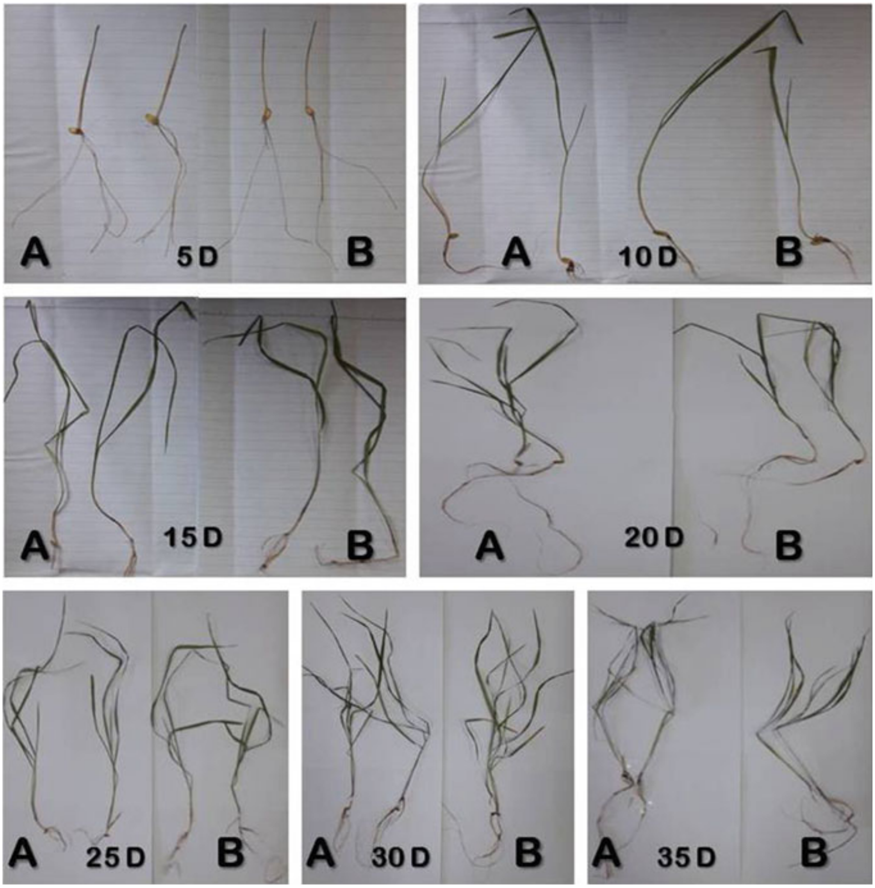

Fig. 1.3 The impact of AgNPs on wheat seedling morphology during 35 days. A: treatment with AgNPs, B: control, D: day (Constructed by L.M. Alnaddaf)

1.5.1 Nutritional Value

The future goals of nanotechnology include high bioactive compound content of secondary metabolites in foods which advantages in improving the nutritional value of industrial crops and stress tolerance (Neme et al. 2021). Plants consider an essential component of the human diet via the supply to our body of vitamins, proteins, minerals, fiber, carbohydrates, lipids, and water (Rivero-Montejo et al. 2021).

The use of NMs has improved the nutritional value of fruits and vegetables without requiring increased consumption by affecting the biochemical and physiological properties of plants (Gomez et al. 2021).

1.5.2 Abiotic Stresses

The soil salinity, water's decrease, and heavy metal increase are the most dangerous to a plant's life cycle which knows as abiotic stresses (Gong et al. 2020).

Stresses adversely affect the plant growth and development via changes in the structural and chemical composition of the plant which lead decreasing in quality and quantity of production. Plants have developed various mechanisms to tolerate these challenges via transferring the stress signals within cells and between cells and tissues, sorting suitable chemical compounds for survival and reproduction, and continuing their growth and development (Rivero-Montejo et al. 2021).

Many reports indicated that when plants are treated with NPs their responses to stresses will be various via influence on biological and metabolism pathways. Also, their role to improve crop tolerance to abiotic stress for instance drought, salinity, and heavy metal stresses (Zohra et al. 2021).

1.5.3 Tissue Culture

A chapter is included to discuss the role of NMs in plant cell and tissue culture. It explains the impact of NMs on in vitro responses. Different NPs in tissue culture media could improve the callus induction, biomass and morphogenetic potential in explants (Barbasz et al. 2016).

NPs play a vital role in various biochemicals, physiological and anatomical routes of tissue culture. NPs improve regeneration, organogenesis, decontamination and secondary metabolite production to protect plant cells and tissues from biotic and abiotic stress. NPs have affected nutrient and protein levels and modulate the expression of certain genes encoded in certain proteins. In addition, it highlights the mechanism affecting callus, and quantitative and qualitative features of calli (Dallavalle et al. 2015).

In addition, it presents an overview of some important applications of NMs in plant tissue cultures such as somaclonal variation, organogenesis, somatic embryogenesis, disinfection, genetic fidelity, and regeneration (Devasia et al. 2020).

1.6 Conclusions and Prospects

This book provides an update on research and development in plant nanotechnology. It covers comprehensively various methods to synthesize NM and its characterization and applications. Moreover, explains the interaction of the NPs with plant cellular mechanisms and macromolecules. The initial phase is interacting NPs with the plant surface lead to adsorption it from the root and penetrate cell wall to move in plants. The second phase begins from the series of different effects at various levels, such as the molecular, biochemical, physiological, morphological and productivity, which it reflects stimulating or inhibiting on the growth and development of the plant.

In addition, the book highlights the implications of NPs in different stages of plant growth and their effect on decreasing or increasing the quality and quantity of production as well as application in tolerating various stresses. Moreover, the book presents the role of NPs in tissue culture and their impacts on the callus physiology, biomass of explants and secondary metabolites according to the type of NPs and their concentrations.

Hence, future research needs to understand the mechanical complexity of interactions NPs with the plant (uptake, translocate, and accumulation) in different parts of a plant. Also discussed in details their effect of different NPs on growth stages in various plant species at the cellular levels.

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Part I
Cellular Mechanisms

Chapter 2
Effect of Nanomaterials on Water and Solutes Translocation in Plants

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Abstract Two decades ago, the discovery of plants' ability to generate engineered nanoparticles (NPs) sparked interest in the subject. Over the past decade, various aspects of the plant-engineered nanomaterials interaction have been acknowledged. The interaction between plants and engineered NPs is one of the fundamental

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factors that will shape the future of nanotechnology. This chapter summarizes recent advances in this area. Afterward entering the soil, many NPs will interact with plants and possibly alter their physiology and food safety. Phytotoxicity is a broadly examined characteristic of the interactions between plants and NPs. It shows that the accumulation of engineered NPs in plants regulates various physiological, biological and biochemical effects on plants. The physiological obstacles to the plant uptake of engineered NPs are discussed. The concept of the rhizosphere and phyllosphere-induced transformation of plants is also explored. The current state of analytical techniques for assessing the uptake of engineered NPs in plants is also addressed. Therefore, the current chapter discusses nanomaterials uptake and transport mechanisms, nanomaterials transformation in rhizosphere and plants, exploring nanomaterials in plants using advanced methods, effects of nano particles on physiological, biochemicals and biological processes and NPs phytotoxicity.

Keywords Nanomaterials · Plants · Phytotoxicity · Water and solutes translocation

2.1 Introduction

The advantages of the recent emergence of engineered nanomaterials (ENMs) are well defined (Abbas et al. 2020). Although ENMs have been produced for decades, wide-scale marketable production has only begun recently. Nanomaterials (NMs) are materials representing at least mono-dimension sized, approximately between one to hundred nanometers (Buzea et al. 2007). It has been described that ENMs were tripled in almost the last decade to reach 1000 consumer products per day (Surendhiran et al. 2020). Although ENMs have demonstrated important aspects in the industry section, it poses a significant improving tool for the manufacture of food and performs a critical role in distinguishing innovative agriculture (Fraceto et al. 2016; Liu and Lal 2015; Mittal et al. 2020; Rai et al. 2018; Sabir et al. 2014; Xin et al. 2020). Frankly speaking, recent knowledge on the effect of NMs on water and solutes translocation in plants remains extremely limited and is not systematic (Avellan et al. 2021; Buriak et al. 2022; Rico et al. 2011; Schwab et al. 2016; Zuverza-Mena et al. 2016). Therefore, ENMs uptake and translocation by plants across water and solutes is a very recent research field (Fiol et al. 2021). Current reports showed that not all ENMs were taken up and translocated to shoot parts by the plant where several factors influence the uptake and translocation processes (Rastogi et al. 2017; Saleh et al. 2021b; Teske and Detweiler 2015) and no trend could be generalized at present (Asare et al. 2012; Becaro et al. 2017; Dumont et al. 2022; Dykman and Shchyogolev 2018; Letchumanan et al. 2021; Mirzajani et al. 2013; Nhan et al. 2015; Rafique et al. 2014; Sadak 2019; Venkatachalam et al. 2017; Wang et al. 2016; Yin et al. 2012).

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Taken up of ENMs through the plant system, it starts translocations and accumulate in different shoot system and consequently the possibility of their reprocess in the environment via different biochemical reactions is increased (Torrent et al. 2020). The unfavorable high concentrations of ENMs in the plant tissues will negatively affect the crop quality, seed germination, photosynthesis, transpiration rates and eventually the crop yield (Hossain et al. 2016). However, a defense system utilizing an enzymatic and/or nonenzymatic approach is a natural aspect for plants to stay alive under ENMs stress conditions (Hossain et al. 2016; Rico et al. 2015). Once the NMs have entered the plant, there are two ways in which they can move through the tissue; (1) the apoplast and (2) the symplast (Lv et al. 2019; Miralles et al. 2012). However, in the case of spray treatments, NMs must overcome the obstacle presented by the epidermis and follow the hydrophilic or lipophilic pathway. The hydrophilic pathway is reached across polar aqueous pores in the epidermis and/or stomata while the lipophilic pathway requires dispersion across cuticular waxes (Attia and Elhawat 2021; Liu et al. 2022; Sathiyabama and Manikandan 2021).

In the last decade, scientists have started to demonstrate the interaction of plant and ENM, their translocation and bio-accumulation and variation as procedures disturbing the ecological consequence of ENMs. Their uptake and bioaccumulation in various plant parts are preferred owing to exposure concentration levels in the range of μg-mg kg^{-1} (Bouguerra et al. 2019). The concentration amounts of ENMs, in both soil and plant, were analytically detected to be very low with lower soil mobility (Darlington et al. 2009). Taking into account the detail which in real conditions, the soil pores developed as a result of several biological factors including root exudates, fungous hyphae and bacteria cells (Zhao et al. 2012b); this little mobility of ENMs demonstrates that: (1) ENMs could be very close to the plant rhizosphere (Gardea-Torresdey et al. 2014; McNear Jr 2013) and (2) the slow revers dependent kinetic reactions are the main process of maintaining plant-ENM accumulation lower in soil (Schwab et al. 2016).

In general, the approach NMs move in plants is very significant as it can provide insight into which parts of the plant they can access as well as potential accumulation points. Though translocation is not necessarily restricted to a specific cell sort and lateral movement of NMs between the phloem and xylem is possible (Banerjee et al. 2019; Murali et al. 2021). In contrast, exposure to NPs through foliar exposure is different from root exposure. The main difference is under foliar exposure, the plant cuticle is protected against the loss of substrate tissue. The uptake of polar and non-polar solutes by the cuticle is achieved through two routes, which are the polar aqueous pores and the diffusion and permeation pathways (He et al. 2022).

Due to unclear physiological and mechanistic factors, research on the uptake and transport of ENPs in plants is quite limited (Gong et al. 2019). Understanding the uptake, translocation and phytotoxicity of ENPs in plants is of critical importance. Analytical measurements of ENPs in real environmental conditions are difficult to achieve at their extremely low concentrations. Therefore, the development of specialized analytical techniques is required to provide quantitative analysis and in situ detection of ENPs (Majumdar et al. 2021). Therefore, the current chapter aims to review information about the interactions between ENPs and plants and the factors affecting their transport and translocation to various parts of the plant.

2.2 Nanomaterials Uptake and Transport Mechanisms

Nanotechnology is a catch-all phrase for materials and gadgets that operate at the nanoscale. In the metric method of measurement, nano equals a billionth and thus a nanometer is one-billionth of a meter. References to NMs, nanodevices and nanopowders simply mean the material or action can be determined in nanometers. To understand the size, a human being's red blood cell is around 2000 nm long, practically out of the nanoscale range (Sharma et al. 2019). Despite the view that nanotechnology is a far-fetched idea with no near-term applications. Nanotechnology has now been recognized as a beachhead in various fields. Most nanotechnologies applications currently depend on nanosized particles. For instance, it produces nanoscale oxides for a wide variety of applications (Fig. 2.1). The investigation community is vigorously pursuing hundreds of applications in NMs and nanobiotechnology (Saleh et al. 2021a). Most near-term applications of nanotechnology are in the shape of NMs. These contain materials such as lighter and stronger nanocomposites, antibacterial NPs and nanostructured catalysts (Usman et al. 2020).

It is a material that can be used in small amounts to promote plant growth and also attain more efficient and sustainable practices in agriculture (Ramírez-Rodríguez

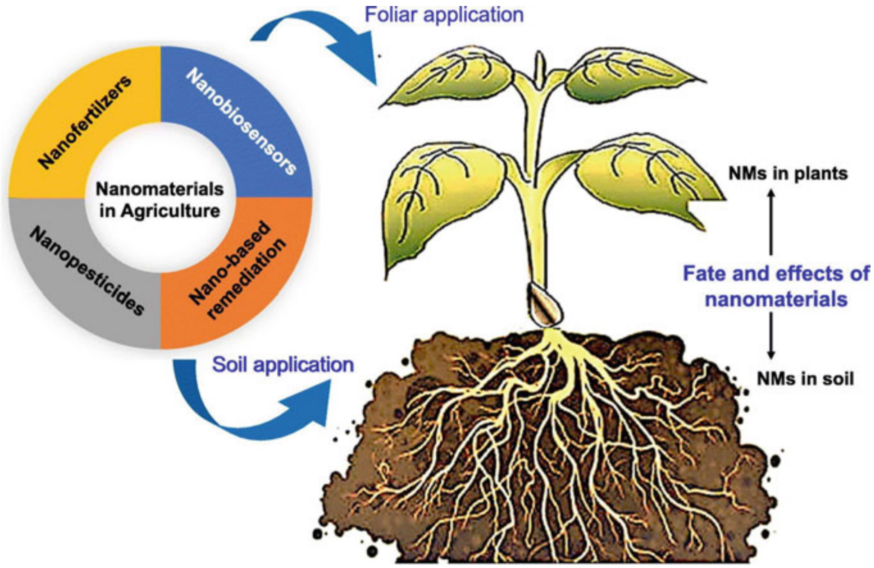

Fig. 2.1 Agriculture and nanotechnology: Situation, issues and future prospects. *Source* Usman et al. (2020)

et al. 2020). These NMs are microscopic particles with at least one dimension within the 1–100 nmsize range (Khan and Upadhyaya 2019). It is used successfully in many agro-production fields (Omara et al. 2019; Salem et al. 2021a, b). It is commendable to point out that the nutrient use efficiency seemed to be lower than 50% for macronutrients in intensive agriculture and this probably results in unsustainable financial and environmental costs. However, this promising technology can improve nutrient uptake by plants and consequently enhance crop productivity while amending very low concentrations of nutrient inputs. Moreover, nano-fertilizers can further improve plant development (Salem et al. 2021a; Sharma et al. 2019). In contrast, the possibility of NMs accumulating and contaminating the environment must be considered in light of their possible damaging impacts on surviving systems, involving plants (Giorgetti 2019). In this concern, some NMs recorded harmful effects on plants (Wan et al. 2019) causing potent oxidative stress to plant leaves and leaf vein chlorosis (Xiao et al. 2019).

Applying some NMs enhances water and nutrient use efficiencies by cucumber under water shortage and salinity stress (Alsaeedi et al. 2019). Similar results were observed when using another nanomaterial as they provoked the greatest oxidative stress reactions (Yusefi-Tanha et al. 2020) and improved the resistance of rapeseed plants to salinity stress (Hezaveh et al. 2019). Several investigations addressing the interaction between NMs and plants have significantly expanded during the past few years.

2.2.1 Uptake and Transport

Numerous factors linked to the nature of the NMs, as well as the plant physiology and the interaction of the NMs with the environment, have an impact on the plant uptake of NMs (Fig. 2.2).

The properties of the nanomaterial will have a significant impact on how it behaves and if the plant can absorb it. There have been studies about the largest dimensions that plants permit for NMs to travel and concentrate inside the cells, often with a size exclusion limit of 40–50 nm. Size appears to be one of the primary barriers to penetration into plant tissues (Taylor et al. 2014). Another element affecting uptake is the type of NMs and its chemical makeup (Rico et al. 2011), while morphology has also been shown in some instances to be a determinant (Raliya et al. 2016). The qualities of the nanomaterial's absorption and accumulation by the plant can be significantly altered and changed by functionalizing and coating the nanomaterial's surface (Judy et al. 2012).

Physiological differences between plant species can lead to changes in NM uptake (Zhu et al. 2012). These studies demonstrated how plants subjected to magnetic carbon-coated, titanium dioxide or gold NMs displayed a variety of absorption and accumulation patterns. The crop species studied belonged to various botanical groups. How a plant will internalize the NMs, however, depends on the application methods. While leaves are designed for gas replacement and have a cuticle that

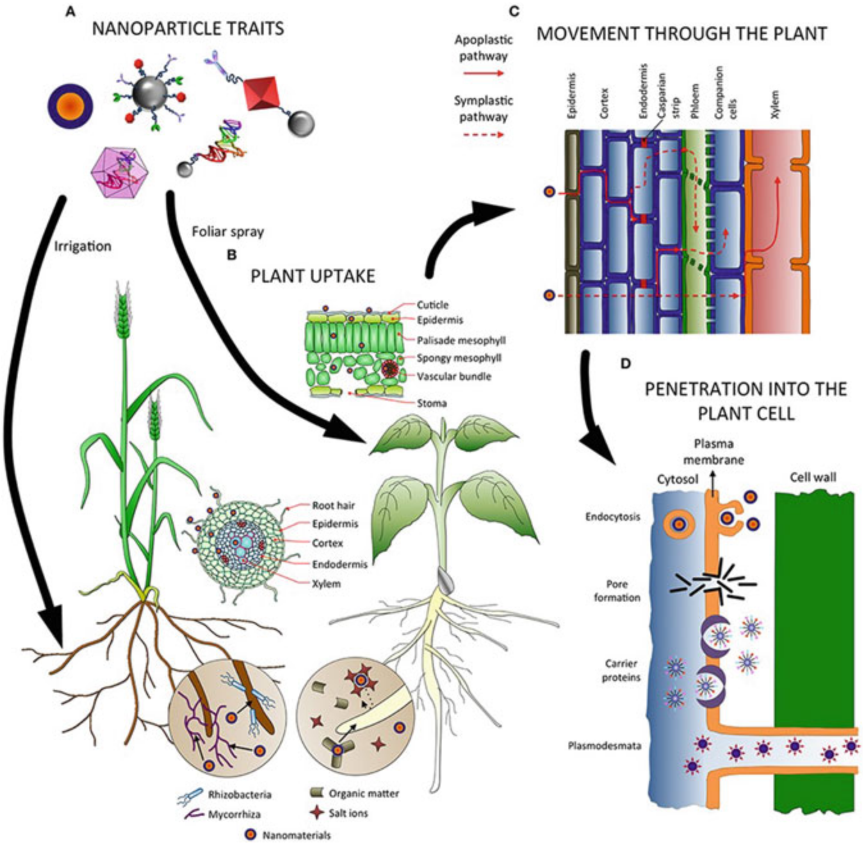

Fig. 2.2 Factors affecting uptake and transport of nanomaterials (NMs) in plants. (A) NMs characteristics affect how they are up taken and translocated in the plant, as well as the treatment method, (B) In the soil, NMs can interact with microorganisms and compounds, which might assist or impede their absorption. Various tissues (epidermis, endodermis) and barriers (Casparian strip, cuticle) must be crossed before attaining the vascular tissues, depending on the entry point (roots or leaves), (C) NMs can follow the apoplastic and/or the simplistic pathways for moving up and down the plant and radial movement for changing from one pathway to the other and (D) Various processes have been proposed for the internalization of NMs inside the cells, such as endocytosis, pore formation, mediated by carrier proteins and through plasmodesmata. *Source* Pérez-de-Luque (2017)

inhibits substances from penetrating, roots specialize in the absorption of nutrients and water (Schwab et al. 2016). However, when nanomaterials interact with other environmental factors, it can change their characteristics and their susceptibility to being digested by plants. For instance, humic acids and other organic matter in the soil might promote stability and, as a result, nanomaterial bioavailability, whereas salt ions may cause precipitation and have the opposite effect. Additionally, the presence of other creatures such as bacteria and fungi, affects how plants absorb nanomaterials, especially if those microorganisms procedure symbiotic relationships with plants, as is the case with mycorrhizal fungi (Perez-de-Luque 2017; Wang et al. 2016).

2.2.2 Movement Inside Plants

After entering the plant, nanomaterials can migrate through tissues in one of two ways; either through, 1) the apoplast or 2) the symplast (Fig. 2.3). In dissimilarity to symplastic transport, which includes the movement of water and other substances within the cytoplasm of adjacent cells through particular structures known as plasmodesmata and sieve plates, apoplastic transport happens outside the plasma membrane through the extracellular spaces, cell walls of adjacent cells and xylem vessels (Sun et al. 2014). The apoplastic pathway is crucial for radial movement inside plant tissues and enables nanomaterials to reach the vascular tissues and the central cylinder of the root, which will then allow them to migrate up the aerial part of the plant (Zhao et al. 2012a).

Nanomaterials can travel toward the aerial section through the xylem after they are within the central cylinder by following the transpiration stream (Sun et al. 2014). The Casparian strip, a barrier to the apoplastic pathway, must be crossed in a symplastic manner via endodermal cells to access the xylem through the root. The Casparian strip can stall and accumulate some nanomaterials (Lv et al. 2015). Utilizing the sieve

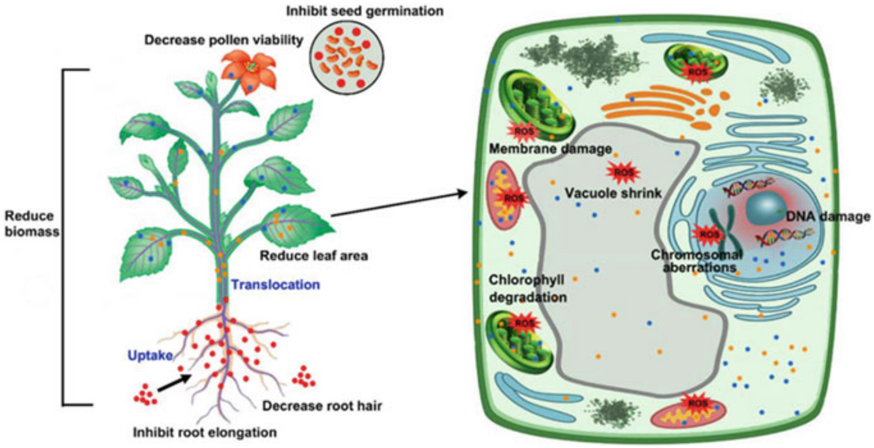

Fig. 2.3 Schematic chart signifying uptake, translocation and main phytotoxicity of silver nanoparticles (AgNPs) in the plant. Normally, AgNPs are taken up by underground tissues (primary roots and lateral roots), then translocated to airborne parts (stem, leaf and flower), where they can decrease biomass, pollen viability and leaf area or inhibit seed germination. At the cellular level, AgNPs enter several organelles, leading to the production of excess reactive oxygen species (ROS), thus affecting cytotoxicity and genotoxicity, for example, membrane damage, chlorophyll degradation, vacuole shrinkage, DNA damage and chromosomal abnormalities. *Source* Yan and Chen (2019)

tube components in the phloem and permitting delivery to non-photosynthetic tissues and organs, another significant symplastic transport is also conceivable (Raliya et al. 2016). Nanomaterials must pass the cuticle's barrier in the event of foliar applications by taking either the lipophilic or the hydrophilic route. The hydrophilic pathway is performed through polar aqueous pores present in the cuticle or stomata, whereas the lipophilic pathway requires diffusion through cuticular waxes. The stomatal channel appears to be the most likely route for nanomaterial penetration because the diameter of cuticular pores has been assessed to be around 2 nm, with a size exclusion maximum of over 10 nm (Zhu et al. 2012). It's crucial to understand how nanomaterials migrate within plants since it can indicate where they might end up and accumulate as well as which areas of the plant they can access. If a sort of nanomaterial, for instance, is transported frequently through the xylem rather than the phloem, it will likely go primarily from the roots to the shoots and leaves rather than downwards, so it should be administered to the roots to get a great distribution in the plant. On the other hand, foliar spraying must be utilized for application if the nanomaterials exhibit good phloem translocation. Another crucial factor is that nanomaterials traveling along the phloem will probably collect in plant organs functioning as sinks (fruits and grains). So, when attempting to prevent additional human or animal consumption of nanomaterials, that is another crucial factor to take into account. However, translocation is not always limited to a particular cell type, and nanomaterials may migrate laterally between the xylem and phloem (Perez-de-Luque 2017). Translocation and accumulation in plant tissues will be significantly influenced by the properties and makeup of the nanomaterials as well as the type of plant involved. For instance, for a similar type of nanoparticle, variances in translocation and accumulation have been reported in numerous plant species, but minute variations in comparable NPs result in various outcomes within a similar plant (Almuhammady et al. 2021; Zhu et al. 2012). According to Cifuentes et al. (2010), wheat and pea have quicker translocation to the aerial portions than sunflower and tomato. However, pea has a higher accumulation of carbon-coated iron NPs in the roots than sunflower and wheat. Zhu et al. (2012) discovered that the roots of ryegrass and radish accumulated greater quantities of gold nanomaterials than those of rice and pumpkin. They also discovered that positively charged gold nanomaterials were also absorbed by roots more quickly than negatively charged ones. While the latter was more effectively transported to the aerial parts. This behavior was attributed to the presence of a negative charge in plant cell walls, which encouraged the buildup of positively charged NPs in tissues and impeded their transport across the plant (Perez-de-Luque 2017). Similar to how it occurs in mammals, nanomaterials typically accumulate in specific tissues and organs (Varna et al. 2012). Specifically, flowers, young leaves fruits and grains are expected to acquire nanomaterials migrating through the vascular system because they act as strong sinks for sap and nutrients (Koo et al. 2015; Lin et al. 2009; Servin et al. 2013). We can take use of that when determining the function of the nanomaterial we want to evaluate. Knowing the nanomaterials' fate is also essential if we don't want them to remain in the body after treatment and be consumed by humans or other animals. Some nanomaterials may be kept in tissues that won't be used after harvesting or may be altered or destroyed by the plant over time (Lv et al.

2015). In the latter instance, it might be salvaged and repurposed for additional use (Liu et al. 2017).

2.2.3 Interaction of NMs with Plant Cells

Nanomaterials need to be ingested by the plant cell and pass through the plasma membrane to go along the symplastic route. Even though such mechanisms are well understood in animal cells and less well-known in plants, nanomaterials can achieve this in different ways (Schwab et al. 2016); Ion channels have been postulated as potential paths for NMs entry into the cell. (a) Pore creation: Certain NMs can disturb the plasma membrane causing the formation of pores for crossing into the cell and extending the cytoplasm without being contained in an organelle (Schwab et al. 2016). However, since these channels are only a few nanometers wide, it is extremely improbable that nanomaterials could successfully cross them without substantial alterations. (b) Carriers: Proteins in the environment, such as those in the cell membrane, can bind to NMs, making them potential carriers for internalization and uptake within the cell. Aquaporins have been recommended as transporters for NMs inside the cell, although they are unlikely to act as channels for NMs penetration due to their small pore sizes, which range from 2.8 to 3.4. This is unless the pore sizes could be manipulated and expanded (Schwab et al. 2016), (c) Endocytosis: By invading the plasma membrane, the NPs enter the cell and create a vesicle that can move to other cell compartments (Etxeberria et al. 2006) and d) Plasmodesmata: These specialized transport mechanisms between cells are another route for nanomaterials to enter a cell. Of course, this requires that the NPs be present in the symplast already, but this method is crucial for translocation across the phloem in plants (Zhai et al. 2014).

2.2.4 Foliar Exposure and Uptake of NPs

On the other hand, exposure to NPs through foliar exposure is different from root exposure. The main difference is that under foliar exposure. The plant cuticle is protected against the loss of substrate tissue. The uptake of polar and nonpolar solutes by the cuticle is achieved through two routes, which are the polar aqueous pores and the diffusion and permeation pathways. Although the sizes of polar solutes are generally smaller than those of nonpolar ones, they can still penetrate through the cuticle (Huang and Keller 2021). The cuticle of NPs must be treated correctly to avoid them from getting damaged. Also, it is worth noting that the first barrier of leaves versus NPs can be easily bypassed in certain circumstances. Through the cuticular pathway, hydrophilic substances can be uptake through the stomatal pathway. The size omission boundary of stomatal apertures for NPs penetration is even not known. This pathway is the merely known route for the foliar uptake of NPs. It has been estimated that its comparable pore radius is greater than 20 nm (Huang and Keller 2021).

Numerous studies have shown that the NPs uptake pathway is supported by the observed accumulation of NPs in leaf stomata (Anjum et al. 2019; Huang and Keller 2021; Majumdar et al. 2021; Shekhawat et al. 2021). Some of these include studies involving the different plant species that exhibited this feature. The uptake of 43 nm NPs was detected by confocal laser scanning microscopy (CLSM) through the stomatal pathway. They also observed the fluorescence in the leaf apoplast after it entered the substomatal cavity (Chavez Soria et al. 2019). The results of these experiments show that the pore radius of the pathway is not as accurate as previously believed. Further studies reveal that the stomatal aperture opening is promoted by the nano-zerovalent iron (nZVI) (Al-Amri et al. 2020; Kim et al. 2019).

The investigation was conducted to study the exfoliant properties of various vegetables, including spinach, collard greens and kale, utilizing single-particle inductively coupled plasma-mass spectrometry (Maswada et al. 2020). The results showed that the NPs exhibited a great degree of water resistance and could be rinsed with water. After reaching the leaf apoplast, NPs could travel long-distance through the vascular system. The long-distance transport of fluid materials in plants happens through the vascular system, which consists of the phloem and xylem conductive tissues. These systems regulate the flow of materials from the top to the bottom of plants (Abobatta 2018). The phloem system is the single pathway for introducing NPs to the plant root. Although many studies have shown the importance of the phloem system for the foliar uptake of NPs, the exact pathway is not known. They discovered that the tiny NPs might penetrate the leaves after the stomatectomy pathway utilizing transmission electron microscopy (TEM). The metal elements were then identified in the roots and shoots (Ahmed et al. 2021). The concentration of Ce was identified by ICP-OES in the tissues of the plants handled with CeO_2 NPs. It was also detected in the roots of the tested plants. They suggested that the presence of water can accelerate the dissolution of $Cu(OH)_2$ NPs by causing leaf exudates to form weak acids (Arif et al. 2018). No evidence was found to support the conclusion that the metal particles identified by TEM in the shoots and roots were from dissolved NPs. Some studies study highlights the importance of using elemental analysis to follow the movement of NPs in plants (Ma et al. 2019; Shende et al. 2015). Some studies presented that the xylem and phloem-based transportation of CeO_2 NPs can be used to reduce CuO NPs from Cu(II) to Cu(III). They also observed that CeO_2 NPs can be transported from shoots to roots across the xylem. Although they can transport up to the xylem, NPs can also transport their products downward through the phloem. This suggests that their products may be transported to the rhizosphere and the phyllosphere (Ma et al. 2019).

2.2.4.1 Phyllosphere Factors Affecting Foliar Uptake

The phyllosphere is home to many pathogenic microorganisms. They can produce compounds that are known as polymeric substances or chemicals that can trigger the dissolution of NPs. The phyllosphere influence on the growth and foliar uptake of non-living organisms (NPs) by plants has not been examined. Likewise, the organ growth stages for plants vary depending on various life levels and the cuticle's undeveloped state may allow NPs to enter leaves (Ma et al. 2019). Some diseases, such as leaf mold, can destroy the protecting structures of leaves for example the cuticle and the mesophyll. This impairs the ability of plants to protect themselves against NPs. After entering the leaf apoplast, NPs can experience long-distance transport across the vascular system. The phloem system allows these organisms to transport downward (Majumdar et al. 2019). The long-distance transportation of fluids in plants happens through the vascular system, which is composed of the xylem and the phloem conductive tissues. The xylem system flow direction is inverted, while the phloem flow direction is straight (Husen 2020). The phloem system is the sole pathway for NPs to foliar uptake from a plant. Although numerous studies have identified the various routes that NPs can take to reach the root, none of these studies have supplied direct indications supporting the phloem translocation pathway (Ye et al. 2018). They discovered that the small NPs could reach the roots using the TEM pathway. They concluded that the metal elements were present in the shoots and roots. They hypothesized that the presence of water could accelerate the dissolution of $Cu(OH)_2$ NPs by causing their exudates to form weak acids (Kolenčík et al. 2021).

2.2.4.2 Possibility of Capturing Atmospheric NPs in Plant Leaves

According to studies, plants aid in the elimination of airborne contaminants such as particulate matter (PM), PM_1, $PM_{2.5}$ and PM_{10} from the air. They contribute to the improvement of air quality by trapping these harmful particles (Tombuloglu et al. 2020; Vera-Reyes et al. 2018). Although laboratory experiments have shown that plants can effectively filter and capture airborne NPs, there is no known study on the nature of these organisms' capture capabilities. The effects of atmospheric NPs on air pollution are studied. This is an imperative stage in the assessment of the various properties of plants as atmospheric NPs (Majumdar et al. 2021; Ye et al. 2018).

2.3 Nanomaterials Transformation in Rhizosphere and Plants

2.3.1 Aspects Impacting Root Uptake of NPs

There are conflicting studies about the impacts of exposure to NPs on the plant's roots. The main reason for this is that the plant uptake is impacted by a variety of aspects, such as particle size, morphology and growth stage (Abd-Elsalam et al. 2020; Das et al. 2018). Particle size is one of the most crucial aspects of NPs' absorption by plant roots (Almuhammady et al. 2021). It is understood that plants have a large particle size (Tamez et al. 2019). Even though tobacco NPs were clumped together on the roots, *Arabidopsis thaliana* roots did not absorb the 18 nm Au NPs. Also, the existence of SiO_2 NPs in the roots of the plant prevented them from being taken up by the roots (Yan and Chen 2019). The experiment revealed that nitrogenous NPs with diameters less than 36 nm were transported throughout the plant tissue. These NPs did not accumulate in the roots and did not translocate to the shoot (Khan 2020). It is difficult to assess the SEL for uptake by roots of NPs due to various factors. One of these is the size of NPs in the rhizosphere varies from that of their initial size (Arif et al. 2018).

Another feature that influences the uptake and transport of NPs in plants is their surface charge. In plants, the mucilage layer of the plant root cap is made up of negatively charged NPs. Negatively charged Au NPs were able to remove the mucilage from the root (Malejko et al. 2021). The stored Au concentrations in the roots tracked the order in which the NPs were deposited. The converse order was got in the shoots, which indicated that the negatively charged NPs were preferentially transported through the vascular system (Kumar et al. 2018a).

In some suitcases, the surface charges of NPs have been reduced because of the formation of nano-coronas by the coating of negatively charged root exudate (Sun et al. 2019). More complex scenarios involve the potential aggregation or dissolution of NPs due to the exudates. These scenarios can also affect their bioavailability and root uptake. Various plant species and plants have various growth stages and their oozes affect the surface charge and size of NPs (Majumdar et al. 2019).

The physiological parameters that affect the uptake of NPs in the soil have not been the subject of many investigations. The interactions among plant species and microorganisms can affect the uptake of NPs (Khan 2020; Zhang et al. 2020). In an instance, a superior percentage of Ce translocation was detected in dicotyledons than in monocotyledons. In addition, the root uptake of gold NPs was also dependent on the species (Kumar et al. 2018a).

Kumar et al. (2018b) found that tobacco roots ingested 10 to 50 nm NPs from wheat using XRF and LA-ICP-MS. Managing agricultural risk requires an understanding of the NPs uptake by plants that varies by species. Adding mycorrhizal fungi encourages the uptake of nutrients and water. It may also affect how well NPs are absorbed into the body. Additionally, exposure to 15 nm Ag NPs may result in the production of secondary metabolites that may influence plant absorption (Noori et al. 2020). Due to the destruction of their physiological barriers, many root disfluencies, including root rot and rhizopus disease, might result in the uptake of NPs by the roots (Yan and Chen 2019).

2.3.2 Integrated Root Uptake and Translocation Pathways of NPs

NPs are initially adsorbed onto the surface when they go to the plant roots. The plant surface has a negative charge, which can trigger the secretion of mucilage or chemicals (Fig. 2.4). This characteristic leads to the accumulation of NPs on the root surface. The root surface cuticle is like the leaf surface cuticle. However, unlike the leaf surface cuticle, the root surface does not have a central cuticle. This indicates that the epidermis of the root hairs may be exposed to NPs. In higher plants, there are two primary channels for nutrient intake and transport. The first is the apoplastic pathway, which entails diffusing into the region between the plasma membrane and the cell membrane after piercing a cell wall. For instance, ryegrass roots frequently contain 20 nmZnO NPs. However, in other plants, such as *Arabidopsis thaliana*, 20–80 nm Ag NPs are also commonly found (Sun et al. 2019). NPs can cause the cell wall to collapse or even alter its size of it. They can also enter the intercellular spaces through physical wounding or disease. The pathway to the endodermis is apoplastic. The only way to prevent NPs from reaching the endodermis is by blocking the Casparian strip. Under specific circumstances, NPs may pass through the apoplast and enter a circulatory system without forming the Casparian strip. This pathway can be observed in the graph below. One important apoplastic channel used by plants to enter the circulatory system is the lateral root junction. To determine whether this mechanism is present in other plants, research is required (Noori et al. 2020).

The symplastic route is a pathway that allows NPs to enter a cell. Two barriers prevent them from moving through the pathway. One is the cell membrane barrier and the other is the cytoplasm barrier (Ye et al. 2018). Although ion channels can work for various ions, they are not selective and are generally not bigger than 1 nm wide. The hypothesis of a cellular internalization pathway involving the use of ion channels has many challenges due to plant physiology (Abobatta 2018). The endocytosis pathway of plant cells is poorly understood. It has been suggested that this process involves the use of endocytosis pathways. Sycamore cells in culture ingest 40 nm and 20 nm polystyrene nanospheres in the fluid phase (Etxeberria et al. 2006; Ye et al. 2018). Additional research demonstrated that the surface characteristics of NPs are critical for the endocytic absorption of plant cells (Abd-Elsalam et al. 2020; Das et al. 2018; Kusiak et al. 2022). The investigators also confirmed that the molecular structure of the NPs plays a role in the endocytic uptake of these particles.

The cell walls of plants separate and contain different cells. The transboundary movement of a cell can be initiated by passing through the cell walls through a network of plasmodesmata (Tan et al. 2018). Numerous findings have confirmed that plasmodesmata (PD) facilitate the passage of non-targeted prion traffic, which in turn facilitates the transfer of biological macromolecules from cell to cell (Hu et al. 2020; Ye et al. 2018). According to various research, plants treated with Ag^+ or Au^+ had black dots of Ag^0 or Au^0 visible in the root cells. This suggests that the black dot was not taken up or assimilated by TEM (Noori et al. 2020). There haven't been any firsthand observations of other NPs inside PD up until this point. These

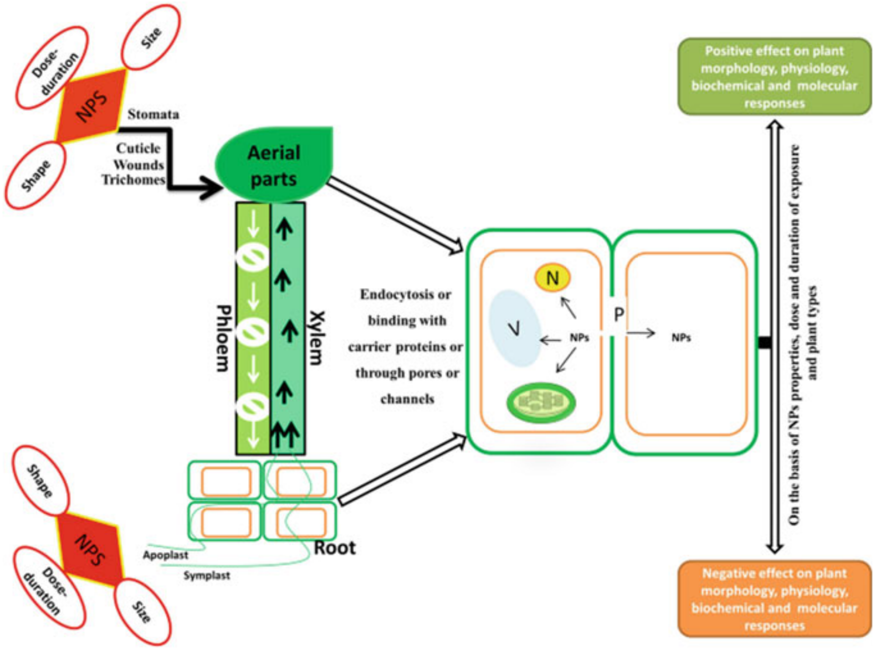

Fig. 2.4 Nanoparticles pathway. *Source* Thounaojam et al. (2021)

NPs may be able to transport proteins across the channel due to the unique chemical characteristics of Ag or Au (Yan and Chen 2019). To better recognize the many mechanisms engaged in plant root uptake and translocation, several physiological and technological difficulties still need to be overcome (Sun et al. 2019).

2.4 Explore Nanomaterials in Plants Using Advanced Methods

Analytical approaches frequently fall short of giving a complete picture of the translocation, modification and uptake of NPs because of the complexity of the plant environment and the size of the NPs in plants. Banerjee et al. (2019) present a comprehensive analysis of analytical techniques that can provide objective information about the NPs in plants.

2.4.1 Analysis in Quantitative Form

Currently, inductively coupled plasma-mass spectrometry (ICP-MS) techniques are used to analyze the metal element content of rare-earth-based NPs to offer quantitative information. However, this method's lateral resolution is not as precise as X-ray microanalysis's (Shende et al. 2015). Single particle ICP-MS can yield strengths of specific NPs of various sizes in plants with the aid of enzyme digestion. This method can also induce the dissolution of the residual NPs (Liu et al 2020). Other techniques that can be used to classify and measure NPs include capillary electrophoresis, field-flow fractionation (FFF) and separation techniques (Ma et al. 2019). FFF-ICP-MS may identify the presence of NPs in soil and water samples even though it is only used to study colloids and NPs. For the most part, these techniques require the digestion and enzymolysis of biological tissues to successfully detect NPs (Ma et al. 2019).

2.4.2 Analysis of Speciation and Location

The most used method for determining the distribution of NPs in plants is TEM. A high-resolution TEM and area electron diffraction combination can be used to measure the NPs' crystal-line structure. The fluorescence-labeled NPs in plant tissues have been identified using FM or confocal laser scanning microscopy. Find out whether the cells have been internalized, it can also be directly observed. High-resolution secondary ion microprobes are used in the secondary ion mass spectrometry method to map the elements in the periodic table. Algae exposed to Ag NP were identified by Ma et al. (2019) using nano-scale secondary ion mass spectrometry (NanoSIMS).

2.4.3 Analytical Methods Based on Stable Isotopes

Fractionation of isotopes is a process that occurs in the chemical and physical transformation of metal elements. This allows researchers to investigate the fate and source of NPs by using special isotope tracers (Das et al. 2018). Unfortunately, it might be difficult for analytical scientists to pinpoint how particles and ions affect the bioavailability of NPs. A new method known as multi-isotope labeling can help solve this issue (Chavez Soria et al. 2019). Various investigations have been released to detect the factors that contribute to the waterborne uptake and flexibility of aqueous zinc oxide (68ZnO) and Zinc oxide NPs (68ZnO NPs). For instance, a significant factor affecting the NPs bioavailability was the solubility of the zinc element (Brenes 2021; Malejko et al. 2021; Pang and Gong 2019; Salem et al. 2021b).

A study revealed that the rapid dissolution of zinc oxides (ZnO) by the end of its lifecycle was the most probable explanation for the absence of cations and particles in soil and water samples. Nano SIMS's superior isotopic resolution has allowed it to map out the samples. But it hasn't been applied to research NPs in living things (Dev et al. 2018). Double stable isotope labeling of NPs can be utilized to evaluate foliar and root exposure of plants in addition to soil samples (Usman et al. 2020). Due to the complexity of the study involved, it is highly recommended that analytical techniques be combined with multiple studies to obtain the most accurate results (Ali et al. 2021).

2.4.4 Physiological, Biochemical and Biological Processes Affected by NPs

Entitled as ENMs, these particles are atomic or molecular aggregates that have small sizes and different physical properties. Because of their diminutive size, cells may readily absorb them (Al-Amri et al. 2020; Alloosh et al. 2021; Alnaddaf et al. 2021). Over the years, various industrial sectors have been using ENMs in their various operations. Their release into the environment has been considered hazardous (Verma et al. 2018). The influences of ENMs on plants have been the subject of numerous investigations. They include evaluations of their potential negative influences on human health and the ecosystem. (Mohammadi et al. 2020). AgNPs are known to be the greatest widely used nanomaterial. Their antibacterial qualities have been extensively utilized in numerous fields, including healthcare, textiles and cosmetics (Ahmed et al. 2021).

Silver NPs are also commonly used as plant growth stimulators and fungicides. They are believed to have complex effects on the cellular membranes and the biochemical processes of plants (Fiol et al. 2021). AgNPs' effects on different plant species are known to vary depending on their various characteristics. For instance, the stability and appearance of the NPs can be impacted by a plant's size, shape and surface area (Zhang et al. 2020). Exposing plants to AgNPs can be performed in a sterile environment to avoid the possible effects of these particles. Numerous investigations have been successfully carried out in the field of in vitro cultures to investigate how different plant species are affected by AgNP-enacted structures (Ali et al. 2021).

Despite their effects on the plant, NMs can also contribute to the development and productivity of plants. They are also known to cause various traits to be formed in the plant. Various enzymes in the plant are known to convert the harmful compounds known as reactive oxygen species (ROS) into useful compounds. These enzymes are also known to contribute to the reduction of oxidative stress and act as biological markers of stress (Dev et al. 2018). Due to the reduced photosynthetic rate caused by stress, the content of chlorophyll, which is a major component of photosynthesis, can decline. This impairs the plant's ability to generate energy. This is one of the reasons why seed germination is a crucial step in plant development (Li et al. 2019). The germination stage is the most sensitive phase of plant development. It can trigger various physiological and biochemical changes in the plant (Rodríguez-González et al. 2019).

Various metrics, including root elongation, are used to evaluate the toxicity of AgNPs, shoot development and germination time. Experiments involving different ENMs have been carried out to examine the impact of these particles on different plant species (Ahmed et al. 2021). One of the experiments revealed that the use of zinc oxide NPs at concentrations of 50–1600 mg/L significantly affected the germination of onion (*Allium cepa* L.). However, the impacts of these particles on the improvement of the plant were not significant. The use of AgNPs did not affect seed germination. However, it inhibited the growth of the plant. The same results were recorded in different plant species (Banerjee et al. 2019).

2.5 NPs Phytotoxicity

In plants, the phytotoxicity caused by NPs is usually triggered by the release of free radicals like hydroxyl radical and hydrogen peroxide. Aerobic metabolism is responsible for the production of ROS, which acts as signaling molecules. When excess ROS levels are observed, they can cause various adverse effects, such as cell death and protein oxidation (Yadav et al. 2020). Previous studies suggest that Plants may experience oxidative stress due to metal and metal-based NPs (Kranjc and Drobne 2019; Rodríguez-González et al. 2019). In ryegrass, particle-dependent production and lipid peroxidation on the cell surface resulted in phytotoxicity. Similarly, a decline in viable cells and an increase in ROS production caused by AgNPs were inversely linked to the treatment period. In addition, the production of the insoluble brown product by the treatment of NPs was demonstrated by the presence of H_2O_2 in the plant roots after being subjected to La_2O_3 and CeO_2 NPs (Yadav et al. 2020).

The treatment with CeO_2 NPs resulted in the buildup of H_2O_2 at a concentration of 35 M, which was greater than the control. However, the experimental results were inconclusive that the treatment caused any oxidative stress or induced ion leakage in the plant (Pang and Gong 2019). The excessive production of ROS can interact with various biological molecules and cause various cellular damages. It can also increase the level of oxidative stress and damage various proteins and DNA.

One of the most damaging processes in the body is the peroxidation of lipids. Malondialdehyde, which is a final product of peroxidation, can cause cell membrane damage. It's been noticed that exposure to various abiotic stresses can increase the level of lipid peroxidation. Over-generation of ROS can also cause the fragmentation of peptide chains, alter the electric charge and make other proteins more vulnerable to proteolysis (Arif et al. 2018; Moghadam et al. 2019). Ag NPs can also cause the formation of thiyl radicals by taking advantage of the cysteine residue. This method allows the NPs to cross-link a disulfide bridge between one thiyl radical and another (Ma et al. 2019; Shende et al. 2015). Twenty-eight proteins have been identified as being engaged in various biochemical and physiological processes after rice (*Oryza sativa* L.) was exposed to Ag NPs. Tombuloglu et al. (2020) assessed how Ag NPs affected the proteome level. They discovered that 16 common proteins in the plant were significantly affected by the degradation of the photosystem and protein degradation. The damage caused by exposure to DNA molecules can affect the encoded proteins in the DNA. In plants, exposure to various environmental stresses can increase the level of DNA degradation. CeO_2 NPs effects on the DNA of *G. max* were also studied.

The damage caused by exposure to DNA can affect the encoded proteins in the cell. CeO_2 NPs with high concentrations can also cause adverse outcomes for the DNA of certain plant species. For instance, López-Moreno et al. (2010) discovered that the concentration of CeO_2 NPs in the plant adversely affected the DNA of *G. max*. For instance, the *Allium cepa* root exhibited negative effects due to the concentration of bismuth oxide NPs. Other research looked into the genotoxic potential of TiO_2 NPs (Ma et al. 2019). According to a study done on the *Allium cepa* root, prolonged exposure to high concentrations of TiO_2 NPs can have genotoxic effects. The plant chromosome and micronuclei were also damaged. Another study revealed that contact with Ag NPs increased ROS production and increased the activity of various antioxidant enzymes (Kolenčík et al. 2021). Since phenolic is produced in plants for their protection from pathogens, they may not be considered detrimental. However, the production of ROS is important for the phytotoxicity mechanism (Jahan et al. 2018). Size, form and solubility are a few variables that may have an impact on how much ROS a plant produces. In a study, scientists investigated the toxicity of three various CeO_2 NPs relative to various sorts of plants. The results revealed that the high concentration of CeO_2 caused by 7 nm can damage the plant membrane (Li et al. 2019). Although the concentration of 25 nm CeO_2 did not vary significantly among the control group and its bulk counterpart, the impacts of shape and size on the plant development were studied. In addition, studies on different plant species revealed that exposure to high concentrations of CeO_2 NPs affected the plant's growth stages (Li et al. 2019). Studies suggest that CeO_2 NPs can be bio-transformed to $CePO_4$ and $Ce(CH_3COO_3)$ by altering their composition. The release of $Ce_3{}^+$ ions from the surface of the plant can also play a crucial role in the toxicity of the chemicals. Liu et al. (2020) revealed that the biotransformation of CeO_2 NPs occurs on the plant root surface instead of in tissues. It also mentioned that phosphate release plays a significant part in the biotransformation of the compounds. A study showed that the phytotoxicity of CeO_2 NPs caused by the presence of phosphates was determined by measuring the number of chemicals in sand culture. In a study, researchers discovered that the concentration of the compounds absorbed by the plant caused its growth to be affected (Shende et al. 2015). Other studies also excluded the toxicity of meta-based NPs from their studies. According to a study on the impacts of CuO NPs on the development of the *Arabidopsis thaliana*, the release of Cu^{2+} ions from these particles can partially exacerbate toxicity (Ma et al. 2019; Shende et al. 2015). Although the seeds of *A. thaliana* were not affected by the toxicity of Ag NPs, the presence of Ag^+ ions affected the plant root elongation. The effects of Ag NPs on the antioxidant system and the water quality of the plant were also studied (Sun et al. 2019). Ag NPs can also cause phytotoxicity by disrupting the plant structure by infiltrating the root surface and reducing the plant chlorophyll content. ZnO NPs can perform photocatalytic reactions, whereby promote the production of ROS (Sun et al. 2019). The phytotoxicity of metal-oxide NPs can be affected by the characteristics of the compounds themselves. For now, the various factors affecting the toxicity of these chemicals must be studied to improve our understanding (Noori et al. 2020).

2.5.1 Phytotoxic of Silica NPs

World demand for SiO_2 NPs has increased significantly due to the rise in the use of these materials in various industries. In 2014, the global production of SiO_2 NPs reached a maximum of over 1,400,000 tons (Majumdar et al. 2019). Aside from being used in cosmetic products, SiO_2 NPs are also utilized for biomedical and biotechnological purposes. Some of these include drug delivery and enzyme immobilization (Ma et al. 2019). Due to the wide use of SiO_2 NPs, their toxic effects have been studied. It has already been known that inhaling silica particles can lead to the development of pulmonary fibrosis and silicosis. SiO_2 NPs are known to have favorable effects on the development and growth of plants. They are also commonly used as fertilizer (Arif et al. 2018; Salem et al. 2021a). Despite their toxicity, numerous research has revealed this. SiO_2 NPs can still provide positive impacts on the growth of plants. For instance, in rice, where high concentrations of Si are required for plant development, the use of SiO_2 NPs with a high concentration can induce seed germination and stimulate root growth. Studies on two different plant species revealed that growing under high-salinity conditions can improve the development and germination of plants (Abobatta 2018; Majumdar et al. 2021).

Because of their major penetrability, SiO_2 NPs are thought to affect the growth of plants. They can reduce the oxidative damage caused using enzymes like superoxide dismutase and catalase (Chandrika et al. 2018). Jahan et al. (2018) discovered that seed pretreatment with high concentrations of SiO_2 NPs can improve the physiology of a plant. They also noted that these chemicals can stimulate the production of bioactive molecules. Si NPs' phytotoxic effects have only really been studied at very high doses. In 2019, researchers discovered that the growth of Arabidopsis plants was decreased and chlorosis was observed when treated with highly charged Si NPs. The negative effects of these chemicals were mainly caused by their effects on pH and the adsorption of macro and micronutrients onto the surface of Si NPs (Pullagurala et al. 2018).

The scientists found that the size of Si NPs produced their impacts on plant roots. Si NPs were not charged and exhibited no phytotoxic properties. The genotoxic and phytotoxic effects of Si NPs were also detected in *A. cepa* seedlings after being exposed to varying doses of the chemicals (Tan 2018). Further analysis revealed that the effects of Si NPs on the root meristems were genotoxic. Although they have beneficial effects on plants, they are usually not toxic (Singh et al. 2018).

2.5.2 Phytotoxicity and Genotoxicity of TiO_2 NPs

Owing to their distinct physical characteristics, TiO_2 NPs are mainly used as the main component of cosmetic products. They are also used in the production of various industrial products such as paints, medical drugs and self-cleaning products (Kolenčík et al. 2021). Also, TiO_2 NPs are considered one of the most toxic chemicals in the environment. Their impact on numerous organisms is still being studied. Generally, they can be observed depending on the concentration, size and plant species tested (Jahan et al. 2018). The production of ROS determines how plants react to TiO_2 NPs and the activation of antioxidant defenses. For instance, when applied at concentrations of up to 4.0%, a mixture of TiO_2 NPs and anatase can stimulate root elongation and seed germination (Ma et al. 2019). According to the study findings that the application of large concentrations of TiO_2 NPs delayed the germination of certain plant species and reduced root length. Further studies revealed that the influence of TiO_2 NPs on the vascular system of *Vibrio narbonensis* plants was caused by their oxidative stress. The results of these experiments indicated that the activation of the plant antioxidant response did not stop the injury caused by the chemicals (Liu et al 2020). The effects of varying concentrations of TiO_2 NPs on the seeds of *V. faba* were negative. The effect was increasingly unfavorable as the TiO_2 NPs size increased. Also, the concentration of bulk TiO_2 had the biggest negative effect on the plant (Ma et al. 2019). Ultrastructure studies revealed that the inner portions of root cells were affected by the effects of TiO_2 NPs. The location of specific antioxidant enzymes and components implicated in the response to oxidative stress was also connected to the effects of TiO_2 NPs on the cellular structure of plants (Liu et al 2020).

Genotoxicity investigations have been performed on various plant species. In addition, the effects of bulk TiO_2 and TiO_2 NPs on human lymphocytes were also investigated. The results of these experiments revealed that the concentration of bulk TiO_2 and TiO_2 NPs affected chromosome abnormalities (Shende et al. 2015). Biochemical analysis of the plant membrane damage caused by TiO_2 NPs and bulk TiO_2 revealed a close correlation between the results of genotoxicity and the amount of TiO_2 exposure that the plant receives. The evaluation of TiO_2 NPs toxicity is complicated by the different forms of TiO_2 and their photocatalytic capabilities (Liu et al. 2020).

An investigation contrasting the effects of anatase and anatase-rutile mixture on different plant species revealed that the former had negative influences on the growth and biomass of the plants. The combination of these chemicals also induced stimulatory impacts on the development of the plant (Liu et al. 2020). The results of the cytotoxic and genotoxic studies supported the different effects of the two forms of TiO_2 NPs which are commonly used in treating plants. The effects of the mixture were greater in terms of delaying the cell cycle and causing it to arrest in the G_1 phase in certain plant species, such as basil. Exposure to varying concentrations of TiO_2 with a large concentration of 21 nm led to higher toxic effects and reduced plant development. Although the impacts of TiO_2 NPs on numerous plant species and concentrations can be studied individually, the complexity of the study requires the use of a few simple and controlled experiments (Ma et al. 2019; Shende et al. 2015).

2.6 Conclusions and Prospects

One of the core issues with nanotechnology is how plants and synthetic ENPs interact. Numerous investigations on the interactions between plants and NPs have been carried out over the previous ten years. The physiological obstacles to NP absorption are discussed and their implications for plant absorption are investigated. The goal of this chapter is to present a thorough study of the knowledge that is currently available concerning the translocation, absorption and transformation of NPs in plants. Also, it directs future investigation. The complexity of the interaction between plants and NPs is evidenced by the various characteristics of these two groups. For instance, the size, shape and structure of NPs are known to affect plant interaction with them. However, many of them have growth-regulating properties that can improve a plant's biomass and nutritional quality. Studies have demonstrated that NPs can translocate through various routes, such as ascending and descending phloem. However, these routes require further investigation to determine the mechanism involved in their cell wall invasion. Although many studies have been conducted in hydroponic setups, it is not possible to replicate the conditions of real ecosystems. Therefore, it is important to design experiments that are based on field-made setups.

The investigations on the absorption, modification and transport of metal-based NPs in the soil-plant system are compiled in this chapter. The size, form, and growth stage of the plant have a major impact on the pathways for the uptake of these organisms. The distribution and transformation of NPs, as well as the surface chemistry, are significantly influenced by the production of root exudates and rhizospheric or phyllosphere microorganisms. The acceptance and acculturation of the NPs by plants may also be impacted by these mechanisms. We still don't fully understand the methods by which these organisms can penetrate and infiltrate the soil-plant system despite the numerous surveys on the uptake and transformation of ENPs. Despite the rise in studies on the consumption and transformation of NPs growing, the complexity of their interactions with the plant remains a challenge to overcome. Therefore, it is important to establish a prioritized list of all the potential applications of these organisms. Further studies on the various characteristics of these organisms are also recommended.

For instance, the mechanism of plant uptake and translocation of ENPs is still not clear. The locations of these objects in the plant where they can transform or accumulate are not known. The effects of varying environmental matrices and the toxicity of the transformation products of ENPs are also studied. Due to the wide exposure of plants to ENPs, their toxicity has been neglected. Additional research is also required to examine their possible impacts on the ecosystem and the human food chain. Stable isotopes, multi-informative approaches, and high spatial resolution are required to enable fundamental research on NP absorption and transport in plants. Furthermore, interactions between plants and NPs are frequently noticed. However, until now, studies on the uptake of natural nano-cells have been neglected. The lack of sufficient data on the environmental impacts of these products and the interactions between plants and NPs are some of the factors that require immediate attention.

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Chapter 3
Response of Plant Photosynthesis to Nanomaterials

Sashi Sonkar, Prakash Kumar Sarangi, Brijesh Pandey, Anand Prakash, and Akhilesh Kumar Singh

Abstract An enhancement in crop productivity is one of the crucial requirements in order to circumvent the increasing food demands globally. Crop productivity may be improved via plant nanobiotechnology that involve the integration of plant biotechnology with nanotechnology. The plant nanobiotechnology exploits the nanoparticles (NPs) for boosting the agricultural plant productivity via increasing growth, development as well as active photosynthesis rate of agricultural plants/crops. The photosynthetic performance is exceedingly susceptible biological activity under abiotic stresses. The photosynthetic process under abiotic stresses results in excessive formation of reactive oxygen species (ROS), which overwhelms the plants native ROS scavenging mechanism. However, the engineered NPs have been found to protect and boost the plants photosynthetic effectiveness by diminishing oxidative stress. However, the impact of NPs on photosynthetic effectiveness is found to varied between plants as well as even within species. They either improve plant photosynthetic effectiveness by enhancing the light-harvesting complexes or block routes through obstructing the electron transport chain. For instance, NPs like single-walled carbon nanotubes (SWCNTs) were found to boost photosynthesis by threefold. In contrast, NPs such as iron oxide as well as silver NPs were reported to impede photosynthesis. Overall, the study of the beneficial impacts of NPs on plant systems

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including photosynthesis is yet inadequate. This chapter provides an overview on the current status regarding the influence of NPs on photosynthesis in plants.

Keywords Light-harvesting complexes · Nanobiotechnology · Nanoparticles · Photosynthesis · Reactive oxygen species

3.1 Introduction

Nanoparticles (NPs) were among the most extensively investigated particles of the twenty-first century, fostering a new area of study known as "plant nanobiotechnology (Al-Khayri et al. 2021). NPs are particles that refer to small size natural or manufactured materials having dimensions ranging between 1 and 100 nm with extremely higher surface to volume area ratio (Mukhopadhyay 2014; Pandey et al. 2018; Porwal et al. 2020; Rani et al. 2020; Singh and Porwal 2020; Singh et al. 2018a; Singh et al. 2020; Porwal et al. 2021; Sonkar et al. 2021a). These NPs can modify their physicochemical characteristics over their parent bulk material (Rastogi et al. 2017). Because of their unique qualities and innovative features, NPs are widely utilized in the development of mankind as well as energy sectors (Nel et al. 2006). NPs may be manufactured from a range of bulk materials, and their activities are determined by their particle shape or/and size as well as chemical components (Brunner et al. 2006). Further, the rate of ingress of NPs into plant cells is determined by their surface features and size. The large size NPs are unable to penetrate the cell or impact cell metabolic processes, whereas smaller NPs enter easily (Kashyap et al. 2015; Sonkar et al. 2021b). However, some huge NPs were observed to create large pores to pass via plant cell walls (Rastogi et al. 2017). There are three sorts of NPs based on their derivation: engineered, accidental, and natural. Natural NPs are those that have occurred from the dawn of geological history and yet are still ubiquitous in the environment (mineral compositions, lunar dust, volcanic dust, and so on) (Monica and Cremonini 2009). Incidental NPs are anthropogenic particles produced by man-made industrial operations such as coal incineration, welding gases, as well as diesel exhaust. Engineered NPs are classified into four categories (Kataria et al. 2019; Singh et al. 2018b):

(a) Composites are NPs that have been blended with other NPs or with bigger bulky materials and come in a variety of shapes such as prisms, rods, tubes, and spheres.
(b) Dendrimers are nano-sized polymers that are made up of branching units that may be customized to conduct certain chemical activities.
(c) Metal-based materials include quantum dots, nano-aluminum, nano-zinc, nano-silver, nano-gold, and nano-scale metal oxides such as aluminium oxide, zinc oxide, and titanium dioxide.
(d) Multi-walled carbon nanotubes (MWCNTs), single-walled carbon nanotubes (SWCNTs), and fullerene are examples of carbon-based materials.

Overall, the development, as well as exploitation of NPs having greater volume to surface area ratio, are presently the key interest of nanotechnology towards boosting the competency of better penetration, interaction as well as reproducibility. This innovative approach is now enabled worldwide scientists to solve/resolve various global issues including food shortage together with agricultural hindrance/obstacles. This is supported by the fact that in the current scenario, plant nanobiotechnology is found to depict great potential in the agricultural field (Wu and Li 2022). For instance, it can enhance stress resistance of plant system through nanozymes mediated hunting of reactive oxygen species (ROS). The nanozymes are those NPs that can imitate antioxidant enzyme activities. It has been observed that cerium oxide NPs treated plants become more resistant/tolerant against salinity (Rossi et al. 2016; Wu et al. 2018; Liu et al. 2021), drought (Djanaguiraman et al. 2018), heat (Wu et al. 2017) as well as cold (Wu et al. 2017). Likewise, NPs like SWCNT enhanced threefold greater photosynthetic performance in the chloroplasts. This is owing to not only the speedy transportation of electrons but also the improved activity of signalling molecules like nitric oxide in plants. The nano-mesoporous silica compounds were also found to enhance the photosynthetic performance (Poddar et al. 2020).

Farming is the foremost occupational backbone of the utmost of developing nations. Further, there is need to enhance agricultural yield by 60% from the 2005–2007 level to nourish a population of about 9 billion by 2050 (Lee 2011; van Ittersum et al. 2016; Porwal et al. 2021). Such rapid growth of population will give rise to severe issues pertaining to water, food as well as energy resources (Marchiol 2018). Consequently, instant sustainable intensification is required towards the enhancement of environmentally friendly agricultural yield in present cultivable land. The speedy rise in worldwide population results in excessive utilization of the limited natural sources like land, water as well as soil has been excessively exploited. This requires eco-friendly-based agricultural development having economic viability. Hence, apart from efforts in breeding programs, farm management as well as cultivation practices, there is a further need for the introduction of innovative approaches like nano-assisted agriculture for overall agricultural improvement (Singh et al. 2015; Prasad et al. 2014). The nano-assisted agriculture/farming has potential towards overcoming the forecasted food scarcity. The evolving area of nano-assisted farming has potential for increasing the plant resistance towards the abiotic stresses apart from improving plant breeding as well as farming (White and Gardea-Torresdey 2018; Pulizzi 2019). Therefore, apart from overcoming food scarcity, plant nanobiotechnology also depicts its considerable potential/impacts in sustainable farming.

Plants are vital for ecosystem functioning as primary producers because they transform energy from the sun into organic materials that may be utilized by later trophic levels of the food chain (McKee and Filser 2016). Plants represent a possible channel for the transportation of NPs (Rico et al. 2011). Further, it can accrue in consumers at various trophic levels along the food chain (Zhu et al. 2008). The rate of NP absorption and its impact on metabolic function as well as growth differ amongst plant species. Additionally, the quantity of NPs influences plant functions such as photosynthesis, growth, and germination. NPs either increase photosynthetic activities in plants and bacteria by enhancing the light-harvesting complexes or block routes by obstructing the electron transport chain (Kataria et al. 2019). Furthermore, NPs play a significant role in plant defense against a variety of abiotic challenges (Khan et al. 2017) by activating antioxidative enzymes that scavenge ROS (Wei and Wang 2013). Under abiotic stresses, photosynthesis is an exceedingly susceptible biological entity, where NPs have been demonstrated to protect the process and boost photosynthetic efficiency by lowering oxidative stress (Siddiqui et al. 2014). Figure 3.1 revealed the potential advantageous interactions of NPs with photosynthetic apparatus in plant system under ultraviolet B (UV-B) radiations as abiotic stress. Nevertheless, the reactions of various plant species to NPs varied (Qi et al. 2013; Giraldo et al. 2014; Barhoumi et al. 2015; da Costa and Sharma 2016; Wang et al. 2016, 2018; Wu et al. 2017; Li et al. 2018; Yanık and Vardar 2018; Ali et al. 2019; Dias et al. 2019; Soleymanzadeh et al. 2020; Swift et al. 2020; Elshoky et al. 2021; Faizan et al. 2021a, b; Rai-Kalal and Jajoo 2021; Rajput et al. 2021). NPs in the cultural medium produce oxidative stress, reduce biomass accumulation, photosynthesis, chlorophyll concentration, shoot length, root length as well as germination including nutrition to agricultural plants. NPs also alter gene expression involved in energy pathways, electron transport chain, cell organization, biosynthesis as well as abiotic and biotic stress responses. The impact of NPs on photosynthesis varied between plants and even within species (Kataria et al. 2019). This chapter throws light on the current status concerning the impact of NPs on plant photosynthesis.

3.2 Interaction of NPs with the Plant Systems

The factors responsible for NPs absorption in plant cells involve plant growth conditions, plant type as well as age. Furthermore, the physicochemical properties of the NPs (such as chemical composition, size, dimension, and stability in solution) were responsible for NP uptake, translocation, and accrual in the plant system (Snehal and Lohani 2018). In general, NPs enter the plant root through the lateral root connections and go to the xylem via the cortex and the pericycle. The plant system's interaction with NPs is mostly based on chemical reactions that result in lipid peroxidation, oxidative damage, ion transport activity, and the formation of ROS. When NPs reach plant cells, they react with carboxyl and sulfhydryl groups, altering protein activity (Kurepa et al. 2009). The transporter or pumps found in the cytoplasmic membrane of the roots primarily controlled nutrient and mineral absorption in plants. In certain circumstances, NPs bind to carrier proteins before passing via ions channels, aquaporins, or endocytosis (Snehal and Lohani 2018). Metals such as silicon in its silicic acid state are mostly taken by plants by diffusion (apoplastic transport). Nonetheless, specialized aquaporin (NIP2) is required for symplastic transport. Xylem is in care of an upwards flow of silicic acid to the aerial tissue system, which includes the shoot and leaves (Deshmukh et al. 2013; Snehal and Lohani 2018). Further, the uptake or absorption of NPs or their aggregates by plant cells is determined by their size, which should be smaller than the pore diameter (5–20 nm) so that they may readily reach the cell membrane after passing through the cell wall (Kumar et al.

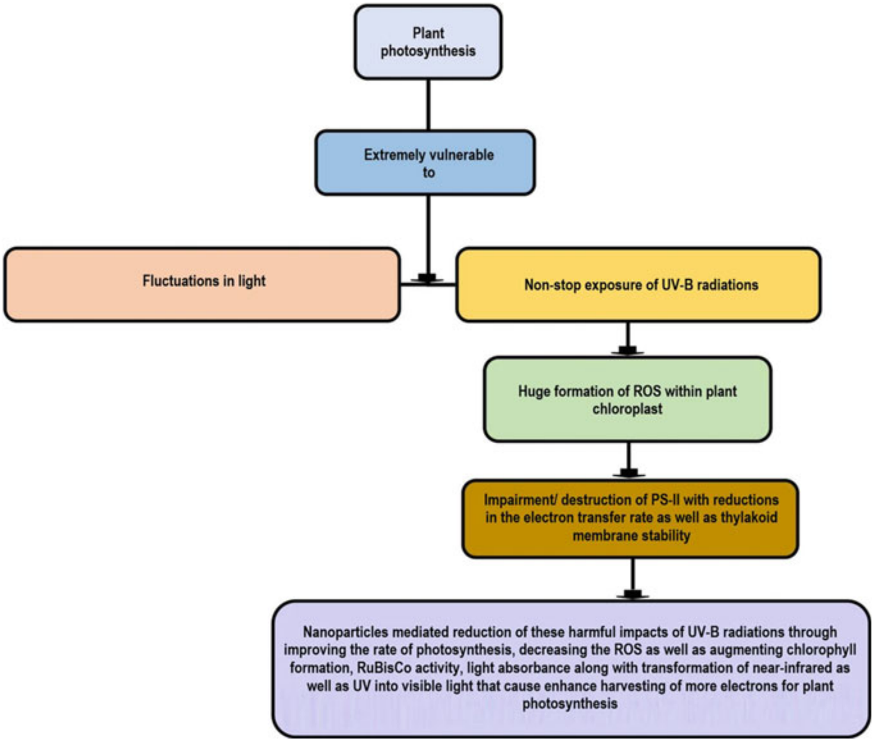

Fig. 3.1 Promising beneficial interactions amongst NPs and plant photosynthetic apparatus under UV-B radiations (Figure constructed by Akhilesh Kumar Singh)

2016). After forming complexes inside membrane transporters or root exudates, the NPs were transferred into the plants. The absorption of NPs by plants has also been documented, either by the stomata or the base of the trichome in leaves (Snehal and Lohani 2018). Following NP penetration into the cell membrane, additional transport occurs via either apoplastic or symplastic routes. NP mobilization following absorption within the plant cell might also be aided by plasmodesmata from one cell to another (Rico et al. 2011; Sanzari et al. 2019).

3.3 Role of NPs in Plant Photosynthesis

Photosynthesis is essentially the only mechanism of energy input in the living world. It is an anabolic process of manufacturing food inside the chlorophyll-containing cells from water as well as carbon dioxide with the assistance of solar light as a source of energy. Recent research is concerned about the participation of NPs and their impact on photosynthetic activity. Some of the favorable impacts of NPs on photosynthesis as well as plant growth have been documented for a variety of plant species (Zarate-Cruz et al. 2016; Cao et al. 2018). To study the rate of energy transformation in plants, the precise interaction of NPs with the molecular as well as ultrastructural components of the plant photosynthetic system must be developed (Tripathi et al. 2017). Hence, the association of NPs with plants and their influences on biological alterations of the photosynthetic system including plant physiological processes, draw attention for assessment and research.

Photosynthesis is more dependent on the structural arrangement of the involved cellular organelle, which is involved in regulating gaseous concentrations inside the cellular system and managing carbon dioxide transit to carboxylation sites (Mediavilla et al. 2001). Factors that influence photosynthetic activity include the regulatory proteins of the thylakoids, presence of photosynthetic pigment (chlorophyll *a* and *b*), activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), carbon dioxide aggregation, adequate grana development, structural stability of mesophyll cells as well as chloroplasts (Sáez et al. 2017). Accordingly, the favorable effects on photosynthesis efficiency with the modification of the aspects liable for photosynthetic yield offer an alternative approach for crop development (Foyer et al. 2017). In addition, solar energy is fundamentally turned into chemical energy via photosynthesis, generating several aspects of the photosynthetic process. NPs can have an influence on photosynthesis in both beneficial as well as harmful ways (Fig. 3.2). It modulates the light-harvesting complex of crops by boosting the reaction, inhibiting the electron transport system as well as altering the function of phosphoenolpyruvate carboxylase, carbonic anhydrase, and RuBisCo thus stopping the metabolic reaction (Kataria et al. 2019). Furthermore, researchers are actively striving to increase agricultural productivity through enhancing plant photosynthetic activity with implanted SWCNTs in chloroplasts. SWCNTs improve the biological detection of signaling chemicals such as nitric oxide and increase the rate of electron transport (Giraldo et al. 2014). Because NPs alter the functionality of photosynthetic components, extensive investigation is necessary to assess the effects of NPs on the final products of photosynthesis. The use of a silicon compound conjugate with photosystem II resulted in a steady photosynthetic reaction for oxygen evolution, which increased the activity of photosynthetic pigments and enzymes. The conjugate might potentially be used in artificial photosynthesis as photo-sensors (Siddiqui et al. 2015).

3.4 NPs and Their Diverse Impact on Plant Photosynthetic Systems

Nonmetallic NPs are biocompatible and less oxidizing. Consequently, they have little or no harmful effects on plant photosynthesis. Metallic NPs, on the other hand, tend to impede photosynthesis by generating ROS, which damages the different photosynthetic machineries (Poddar et al. 2020). The stimulated reactions generated by NPs varied amongst plant species as depicted in Table 3.1.

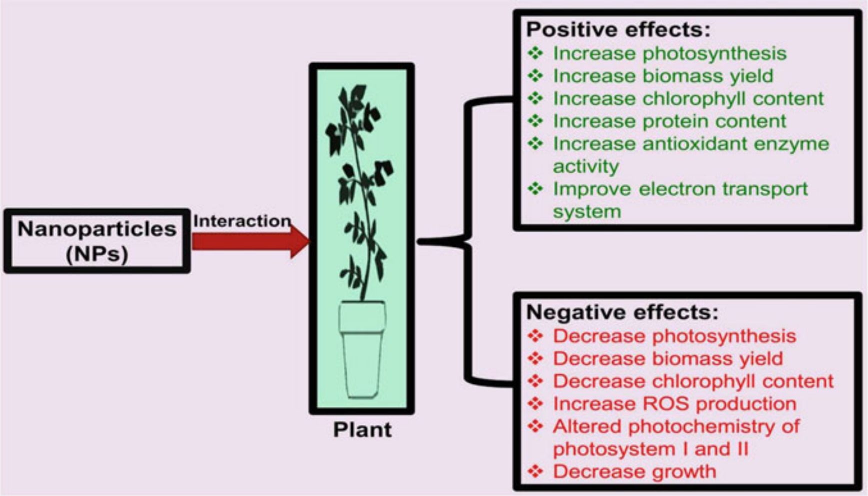

Fig. 3.2 Various positive and negative impacts of NPs on a plant's photosynthetic system (Figure constructed by Sashi Sonkar)

Qi et al. (2013) investigated the impact of TiO_2 NPs on the photosynthetic rate of tomatoes under exposure to mild heat stress. The photosynthetic and transpiration, as well as water conductance, get improved with the treatment of TiO_2 NPs. TiO_2 NPs not only boosted the regulation of photosystem II energy dissipation but also decreased the non-regulated photosystem II energy dissipation under mild heat stress. These findings suggest that TiO_2 NPs promote photosynthesis in leaf tissue under mild heat stress. Giraldo et al. (2014) reported that SWCNTs passively transport and irreversibly localize within the lipid envelope of extracted spinach chloroplasts, increasing maximum electron transport rates as well as promoting photosynthetic activity over three times higher than the control. Through a mechanism compatible with increased photoabsorption, the SWCNT and chloroplast assemblies also permit higher rates of leaf electron transfer in vivo. Delivering SWCNT-nanoceria [cerium oxide (CeO_2) NPs] or poly(acrylic acid)-nanoceria (PNC) complexes considerably reduces ROS concentrations inside removed chloroplasts. The toxicity of superparamagnetic iron oxide nanoparticles (SPION) in *Lemna gibba* L. plants subjected for 7 days to Fe_3O_4 (SPION-1), $Co_{0.2}Zn_{0.8}Fe_2O_4$ (SPION-2) or $Co_{0.5}Zn_{0.5}Fe_2O_4$ (SPION-3) at 0, 12.5, 25, 50, 100, 200, or 400 $\mu g\ mL^{-1}$ was examined. At less than 400 $\mu g\ mL^{-1}$ of SPION, toxicity was shown by a larger production of ROS, impairment of photosystem II activities, and reduction in chlorophyll content as well as suppression of growth rate. The exposure of SPION suspensions to *L. gibba* induced many changes to the entire plant cellular system, which might be attributed to both NP and metal ion absorption in the soluble fraction. The findings show that the SPION has a complicated toxic mode of action on the entire plant system, affecting its viability (Barhoumi et al. 2015). On the other hand, da Costa and Sharma

Table 3.1 Beneficial and detrimental effects of NPs on plant photosynthetic systems

Type of plant species	Type of NPs treatment	Response	References
Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.)	Ag NPs	Severely limit chlorophyll production and plant development, as well as induce oxidative damage	Li et al. (2018)
Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.)	CeO_2 NPs	Increase photosynthesis by allowing for faster RuBisCo carboxylation	Wu et al. (2017)
Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.)	ZnO NPs	Inhibit the expression of photosystem structural genes and chlorophyll biosynthesis genes, resulting in the inhibition of chlorophyll biosynthesis and a decrease in photosynthesis effectiveness in the plants	Wang et al. (2016)
Barley (*Hordeum sativum* L.)	ZnO NPs	Diminish photosynthetic activity and impair the structural organization of the photosynthetic machinery	Rajput et al. (2021)
Mung bean (*Vigna radiata* (L.) Wilczek)	Carbon dots (CDs)	Promotes photosynthesis and plant growth. Increase electron transfer in photosystem, RuBisCo activity, and chlorophyll concentration	Wang et al. (2018)
Pea (*Pisum sativum* L.)	ZnO-Si NPs and ZnO NPs	Reduce the deleterious effects of salt stress on the membrane integrity, stomata closure, pigment content, and photochemistry of photosystems I and photosystems II	Elshoky et al. (2021)
Rice (*Oryza sativa* L.)	CuO NPs	Decreases the concentration of photosynthetic pigment, the maximum quantum yield of photosystem II photochemistry, stomatal conductance, transpiration rate, and photosynthetic rate	da Costa and Sharma (2016)
Rice (*Oryza sativa* L.)	ZnO NPs	Improves mineral nutrient content, antioxidant enzyme activity, protein content, photosynthesis, and biomass	Faizan et al. (2021b)

(continued)

Table 3.1 (continued)

Type of plant species	Type of NPs treatment	Response	References
Spinach (*Spinacia oleraceae* L.)	Single-walled carbon nanotubes (SWCNTs)	Improves the antioxidant system, photoabsorption, and electron transport in chloroplasts	Giraldo et al. (2014)
Strawberry (*Fragaria* × *ananassa* Duch. cv. 'Gaviota')	Se NPs	Improves antioxidant apparatus, salicylic acid, photosynthesis, and ion hemostasis under salt stress	Soleymanzadeh et al. (2020)
Swollen duckweed (*Lemna gibba* L.)	Superparamagnetic iron oxide nanoparticles (SPION)	Effecting viability and shut down of whole photosynthesis process to a huge extent. Higher production of ROS, impairment of photosystem II activities, and reduction in chlorophyll content as well as suppression of growth rate	Barhoumi et al. (2015)
Tomato (*Lycopersicon esculentum* Mill.)	TiO_2	Increased photosynthesis through managing energy dissipation, which resulted in leaf cooling by increasing stomatal opening	Qi et al. (2013)
Tomato (*Lycopersicon esculentum* Mill.)	ZnO NPs	Increase photosynthetic features, chlorophyll content, leaf area, biomass, root length, and shoot length of tomato. Reduces the deleterious effects of salt stress on plant development and increases protein content as well as antioxidative enzyme activity such as catalase, superoxide dismutase, and peroxidase under salt stress	Faizan et al. (2021a)
Wheat (*Triticum aestivum* L.)	CDs	Enhances photosynthesis and boosts crop output, resulting in an 18% upsurge in grain production	Swift et al. (2020)

(continued)

Table 3.1 (continued)

Type of plant species	Type of NPs treatment	Response	References
Wheat (*Triticum aestivum* L.)	Si NPs	Improves leaf gas exchange characteristics and chlorophyll *a* and *b* concentrations while decreasing oxidative stress in leaves as evidenced by increased peroxidase and superoxide dismutase abilities and reduced electrolyte leakage in the leaf	Ali et al. (2019)
Wheat (*Triticum aestivum* L.)	TiO_2 NPs	Reduction in both the light-independent and light-dependent phases of photosynthesis, as well as a decrease in chlorophyll content, the maximum and effective efficiency of photosystem II, starch content, intercellular CO_2 concentration, stomatal conductance, transpiration rate, and net photosynthetic rate	Dias et al. (2019)
Wheat (*Triticum aestivum* L.)	ZnO NPs	Increase seed water intake, which resulted in increased α-amylase activity. The concentration of photosynthetic pigments (total chlorophyll, chlorophyll *b*, and chlorophyll *a* content) was dramatically increased	Rai-Kalal and Jajoo (2021)
Wheat (*Triticum aestivum* L. cv Demir 2000)	Al_2O_3 NPs	Triggers oxidative stress in plants and damages photosynthetic pigment systems. Reduces catalase activity while increasing proline content, lipid peroxidation, superoxide dismutase activity, and hydrogen peroxide content	Yanık and Vardar (2018)

(2016) evaluated the effects of copper (II) oxide NPs (CuO NPs) on the biochemical and physiological behavior of rice plants. At high concentrations of CuO NPs, the biomass, root and shoot length, and germination rate were decreased, whereas Cu absorption in the shoots and roots improved. The increment of CuO NP was documented in the cells, particularly in the chloroplasts, and was associated with a decrease in the number of thylakoids per grana. The concentration of photosynthetic pigment, the maximum quantum yield of photosystem II photochemistry, stomatal conductance, transpiration rate, and photosynthetic rate, all decreased with no photosystem II photochemical quenching at 1000 mg L^{-1} of CuO NPs. Increased proline as well as malondialdehyde levels, indicated osmotic and oxidative stress. Superoxide dismutase as well as ascorbate peroxidase expression levels, were similarly enhanced. Their findings indicated the detrimental impact of Cu buildup in roots and shoots, which resulted in the loss of photosynthesis. Zinc oxide NPs (ZnO NPs) were shown to be toxic to a variety of plant species. Likewise, Wang et al. (2016) investigated the impact of ZnO NPs on *Arabidopsis* plant photosynthesis and biomass accumulation. Treatment with 300 and 200 mg L^{-1} ZnO NPs lowers growth by 80 as well as 20%, correspondingly over untreated system. Chlorophyll a and b content was reduced by more than 50%, although carotenoid content was mostly unaltered in *Arabidopsis* plants treated with 300 mg L^{-1} ZnO NPs. Furthermore, in 300 mg L^{-1} ZnO NPs-supplemented plants show the transpiration rate, net photosynthesis, leaf stomatal conductance, and intercellular carbon dioxide concentration rate were all lowered by more than 50%. The expression levels of chlorophyll synthesis genes including *magnesium-chelatase subunit D (CHLD), Mg-protoporphyrin IX methyltransferase (CHLM), copper response defect 1 (CRD1), chlorophyll synthase (CHLG), and chlorophyll a oxygenase (CAO)*, as well as photosystem structure gene such as *photosystem I subunit K (PSAN), photosystem I subunit K (PSAK), photosystem I subunit E-2 (PSAE2)*, and *photosystem I subunit D-2 (PSAD2)* were reduced about five-folds in 300 mg L^{-1} ZnO NPs treated plants, according to quantitative analysis using reverse transcription-polymerase chain reaction. On the contrary, enhanced expression of numerous carotenoid synthesis genes, including *zetacarotene desaturase (ZDS), phytoene desaturase (PDS), phytoene synthase (PSY), and geranyl geranyl pyrophosphate synthase 6 (GGPS6)*, was found in plants treated with ZnO NPs. These findings suggest that the toxicological effects of ZnO NPs observed in *Arabidopsis* were most likely caused by the inhibitory activities of the expression of photosystem structural genes as well as chlorophyll biosynthesis genes, resulting in the inhibition of chlorophyll biosynthesis with a decrease in photosynthesis effectiveness in the plants. Wu et al. (2017) revealed that nanoceria increase photosynthesis and ROS scavenging in *Arabidopsis thaliana* (L.) Heynh. when exposed to high levels of light (2000 mol m^{-2} s^{-1} for 1.5 h), heat (35 °C for 2.5 h), and dark chilling (4 °C for 5 days). Non-endocytic mechanisms transfer PNC into chloroplasts. PNC with a low Ce^{3+}/Ce^{4+} ratio (35%) lowers leaf ROS levels by 52%, including hydroxyl radicals, superoxide anion, and hydrogen peroxide. When plants with PNC were subjected to abiotic stress, they showed an increase in RuBisCo carboxylation (61%), carbon absorption (67%), and quantum yield (19%) of PS II over plants without NPs. However, PNC with a high $Ce3^{+}/Ce^{4+}$ ratio (60.8%) increase leaf ROS levels and do not protect photosynthesis from oxidative damage during abiotic stress. Li et al. (2018) explores the interactions of silver NPs (Ag NPs) and diclofop-methyl (DM) on the antioxidant system, photosynthesis, and physiological morphology of *Arabidopsis thaliana* (L.) Heynh. Treatment with Ag NPs (0.5 mg L^{-1}), on the other hand, was shown to severely limit chlorophyll production and plant development, as well as induce more severe oxidative damage in plants than the effects reported in a hydroponic solution containing both Ag NPs and DM. Meanwhile, relative transcript levels of photosynthesis-related genes (*pgrl1B, pgrl1A, rbcL,* and *psbA*) in the combined group were found to be somewhat higher than transcript levels in the Ag NPs group, to sustain ATP production at normal levels to heal light damage. Wang et al. (2018) investigate the effects of carbon dots (CDs) on photosynthesis and plant growth of mung bean. A dose–response impact was observed on biomass stem elongation, root elongation, and mung bean sprout growth. CDs at optimum levels also improve the seed moisture levels and root vitality which may have aided plant development and growth. Furthermore, mung bean sprouts treated with CDs had a rise in carbohydrates content (21.9%) as compared to the control condition. They postulated that the rise in carbohydrates was due to the involvement of CDs in photosynthesis. Further research demonstrated that CDs could improve photosystem activity by increasing the transfer of electrons. Other important photosynthetic parameters, including RuBisCo activity and chlorophyll concentration, are similarly affected by CDs treatment. These results hold a lot of potential for agricultural productivity and biological study. Yanık and Vardar (2018) evaluated the impact of aluminium oxide NPs (Al_2O_3 NPs) on wheat (*Triticum aestivum* L. cv Demir 2000) in which wheat roots were treated to varying concentrations of Al_2O_3 NPs (5, 25, and 50 mg mL^{-1}) for 96 h. The impacts of Al_2O_3 NPs were investigated using a variety of metrics, including anthocyanin content, photosynthetic pigment, total proline, lipid peroxidation, catalase, and superoxide dismutase activity, and H_2O_2 concentration. In comparison to the control, Al_2O_3 NPs induced a dose-dependent reduction in catalase activity while increasing proline content, lipid peroxidation, superoxide dismutase activity, and H_2O_2 content. Furthermore, at the dosage of 50 mg mL^{-1}, anthocyanin, carotenoids, chlorophyll a, and total chlorophyll content decreased. Finally, after 96 h, Al_2O_3 NPs triggered oxidative stress in wheat. Similarly, Ali et al. (2019) studied the effects of silicon NPs (Si NPs) on wheat (*Triticum aestivum* L.) growth under heavy metal stress of cadmium as well as cadmium (Cd) accumulation in grains. The results revealed that Si NPs considerably enhanced, compared to the control, the dry biomass grains (27–74%), spike (25–69%), roots (11–49%), and shoots (10–51%) in soil-applied and by 31–96%, 34–87%, 14–59%, and 24–69% in foliar spray Si NPs, correspondingly. Si NPs improved leaf gas exchange characteristics and chlorophyll *a* and *b* concentrations while decreasing oxidative stress in leaves as evidenced by increased peroxidase and superoxide dismutase abilities and reduced electrolyte leakage in leaf under Si NPs treatments over control. As compared with the control, the soil-applied Si NPs reduced the Cd concentrations in grains roots, shoots, and by 22–83%, 10–59%, and 11–53%, respectively, whereas the foliar spray of Si NPs reduced the Cd contents in grains, roots, and shoots by 20–82%, 19–64%, and

16–58%, correspondingly. Si concentrations in shoots as well as roots, rose considerably after foliar and soil Si NP treatment over control. Their findings revealed that Si NPs might increase wheat output but also lower Cd contents in grains. Dias et al. (2019) examine the impact of titanium dioxide NPs (TiO_2 NPs) on photosynthesis in wheat (*Triticum aestivum* L.). The results exhibited a reduction in both the light-independent and light-dependent phases of photosynthesis, as well as a decrease in chlorophyll *a* content, the maximum and effective efficiency of photosystem II, starch content, intercellular CO_2 concentration, stomatal conductance, transpiration rate, and net photosynthetic rate. However, no changes in RuBisCo activity, non-photochemical, and photochemical quenching levels, or total soluble sugar content were reported. Their findings support the hypothesis that induced degradation in chlorophyll *a* concentration hampered electron transport through photosystem II and that stomatal constraint hampered CO_2 uptake. The decrease in starch concentration appears to be a result of its breakdown as a method to keep total soluble sugar levels stable. As a result, it was claimed that photosynthetic-related endpoints are sensitive and relevant biomarkers for determining TiO_2 NP cytotoxicity. Soleymanzadeh et al. (2020) explored the impact of selenium NPs (Se NPs) (10 and 100 μM) on phenylpropanoids, antioxidant system, ion homeostasis, and photosynthetic efficiency in strawberry (*Fragaria* × *ananassa* Duch. cv. 'Gaviota') subjected to salinity stress. Salinity caused a decrease in Ca and K contents and a rise in Na concentration, which were alleviated by the administration of Se NPs. Furthermore, the Se NPs application at 10 μM reduced the NaCl-induced lesion to PS II performance, which contributed to an enhancement in water-splitting complex function under salt stress. Exposure to Se NPs at a concentration of 100 μM caused mild stress, as measured by rises in lipid peroxidation rate and hydrogen peroxide. Under salinity conditions, the Se NPs 10 M treatment boosted the amount of phenylpropanoid derivatives (caffeic acid, catechin, and salicylic acid) and catalase activity while decreasing the content of oxidants. Consequently, using Se NPs at the appropriate concentration can be an efficient way to treat indications of salt stress by improving antioxidant apparatus, salicylic acid (a critical signaling defense hormone), photosynthesis, and ion hemostasis. Swift et al. (2020) investigate the uptake of carbon dots in *Triticum aestivum* L. 'Apogee' (dwarf bread wheat) enhances photosynthesis and boosts crop output, resulting in an 18% upsurge in grain production. The glucose-functionalization improves NPs absorption, photo-protection, and pigment synthesis, resulting in higher yields. This demonstrates the potential of a functional nanomaterial for enhancing photosynthesis as a means of increasing agricultural output. Elshoky et al. (2021) evaluate the effects of zinc oxide NPs (ZnO NPs) coated with a silicon shell (ZnO-Si NPs) and bare (ZnO NPs) on the growth of *Pisum sativum* L. under salt and physiological stress conditions. The experimental results showed that foliar spray with ZnO-Si NPs and 200 mg L^{-1} ZnO NPs did not affect the functions of both photosystems, membrane integrity, and stomata structure under physiological conditions, whereas 400 mg L^{-1} ZnO-Si NPs had a positive effect on the photochemistry of photosystem I and effective quantum yield of photosystem II. On the alternative, minimal phytotoxic effects were seen following spraying with 400 mg L^{-1} ZnO NPs, which was associated with an increase in non-photochemical quenching and promotion of the cyclic electron flow around the photosystem I. The findings also revealed that both types of NPs (except 400 mg L^{-1} ZnO NPs) reduce the deleterious effects of 100 mM NaCl on the membrane integrity, stomata closure, pigment content, and photochemistry of photosystems I and II. The protective effect was greater after spraying with ZnO-Si NPs than after spraying with ZnO NPs, which might be attributed to the existence of a Si covering shell. Faizan et al. (2021a) examines the impact of zinc oxide NPs (ZnO NPs) in the modulation of salt tolerance in tomato (*Lycopersicon esculentum* Mill.). Their findings revealed that foliar spraying ZnO NPs at different concentrations, namely 10, 50, and 100 mg L^{-1} in the presence or absence of NaCl (150 mM), significantly increased photosynthetic attributes, chlorophyll content, leaf area, biomass, root length, and shoot length of tomato. Furthermore, the use of ZnO NPs reduces the deleterious effects of salt stress on plant development and increases protein content as well as antioxidative enzyme activity such as catalase, superoxide dismutase, and peroxidase under salt stress. Finally, ZnO NPs have a vital function in the reduction of NaCl toxicity in tomato plants. As a result, ZnO NPs can be employed to improve tomato development and alleviate the negative effects of salt stress. Faizan et al. (2021b) investigates the effects of 50 mg L^{-1} zinc oxide NPs (ZnO NPs) on plant growth, photosynthetic activity, elemental status, and antioxidant activity in rice (*Oryza sativa* L.) under cadmium stress. However, under cadmium toxicity, rice plants fed with ZnO NPs showed significantly enhanced root dry weight (12.24%), shoot dry weight (23.07%), root fresh weight (30%), and shoot length (34%). Furthermore, the ZnO NPs treatment has beneficial impacts on photosynthesis. ZnO NPs also significantly reduced cadmium-induced increases in malondialdehyde and hydrogen peroxide contents. Biochemical and physiological analysis revealed that ZnO NPs increased the enzymatic activities of superoxide dismutase (59%), and catalase (52%) as well as proline (17%), all of which metabolize ROS; these increases corresponded to changes in malondialdehyde and hydrogen peroxide accretion after ZnO NPs implementation. Eventually, the application of ZnO NPs to rice leaves improves mineral nutrient content, antioxidant enzyme activity, protein content, photosynthesis, biomass, and reduces cadmium levels. This is mostly owing to the decreased oxidative damage using ZnO NPs. Rai-Kalal and Jajoo (2021) indicate that seed priming with zinc oxide NPs (ZnO NPs) has a substantial favorable influence on seed vigor index and germination performance in wheat cultivar H-I 1544 when compared to hydroprimed and unprimed (control) seeds. Furthermore, nanopriming increased seed water intake, which resulted in increased α-amylase activity. The concentration of photosynthetic pigments (total chlorophyll, chlorophyll b, and chlorophyll a content) in nanoprimed plants was dramatically increased. A significant drop in the activity of superoxide dismutase, catalase, peroxidase, and the degree of lipid peroxidation was detected, which might be attributable to lower ROS levels in nanoprimed plants compared to controls. Rajput et al. (2021) evaluate the toxicity of zinc oxide NPs (ZnO NPs) on spring barley anatomical and physiological indicators. ZnO NPs hindered development by changing chlorophyll fluorescence emissions and producing trichome and stomatal morphological deformations, modifications to cellular structures, particularly abnormalities in the chloroplasts, and disruptions to the thylakoid and grana organizations. The number of chloroplasts per cell in barley leaf cells treated with ZnO NPs was lower as compared to control. Zn content buildup in plant tissues caused by ZnO NPs was demonstrated to diminish photosynthetic activity and impair the structural organization of the photosynthetic machinery.

3.5 Conclusion and Prospects

There is a requirement for considerable enhancement in crop productivity to meet food scarcity. The nano-aided farming/agriculture has potential to circumvent food scarcity. NPs offer potential strategies towards the enhancement of plant photosynthesis, which in turn can increase crop productivity. This is supported by the fact that NPs can be exploited for transformation of near-infrared as well as UV into visible light. This is expected to cause enhanced harvesting of more electrons for plant photosynthesis, particularly in light inadequate conditions and, thereby enhancing photosynthetic performance. However, it is yet to be completely exploited. Metallic NPs often reduce the rate of photosynthesis by causing oxidative stress inside the chloroplast. A non-metallic nanoparticle increases photosynthesis by increasing the rate of electron transport inside the chloroplast and provides a defense system against oxidative stress. Considering this, there is need to carefully design/engineer NPs that may lead to a generation of more effective NPs with positive impacts on plant photosynthetic performance. In addition, more research is required to investigate the process of operation of NPs, their interactions with biological molecules, and their influence on regulating the expression in plant species. In addition, there are new concerns that arise to address the biological consequences of NPs to fill the significant gaps in research of NPs phytotoxicity and many unsolved problems. Based on these considerations, it is critical to establish both the deleterious and beneficial effects of NPs on photosynthetic activities in plant species. Plant resistance to abiotic stress is improved by NPs, although the processes underlying this response are still not fully known. More research at the molecular level is required to comprehend the role and importance of NPs at the subcellular level.

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Chapter 4
Impact of Nanomaterials on Chlorophyll Content in Plants

Luis Páramo, Humberto Aguirre Becerra, José Emilio Ramírez Piña, José Antonio Cervantes Chávez, Ana A. Feregrino-Pérez, and Karen Esquivel

Abstract Nanomaterials (NMs) and nanostructured materials (NSMs) possess specific physicochemical characteristics suitable for different applications in medicine, pharmaceuticals, biotechnology, energy, cosmetics, electronics, environmental remediation, and agricultural processes. Due to their size and surface reactivity, these compounds can interact and move through cells and intracellular structures, interfering with the metabolic pathways of plants. Depending on the type of NM or NSM, dose, and exposure time, the effect on plants can be positive, presenting beneficial changes in plant growth and development or harmful, toxic, or even lethal. The changes in different vegetal species produced by NMs or NSMs also depend on their nature, concentration, crystallinity, surface area, morphology, and type of application (through the irrigation system or foliar). The response using this kind of materials on plants can be related to changes in the photosynthesis process, photochemical reactions, quantum yield, and photosynthetic pigments. This chapter recapitulates recent results and studies showing that NMs and NSMs can improve

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photosynthetic efficiency, suggesting that photosynthesis can be nanoengineered for harnessing more solar energy to achieve sustainable horticulture.

Keywords Chlorophyll · Nanomaterials · Nanostructured materials · Plant tissue · Sustainable agriculture · Toxicology

4.1 Introduction

The conventional method to enhance food production in agriculture includes chemicals like pesticides, herbicides, and fertilizers. Most pesticides and herbicides are used to combat biotic stress with major side adverse effects on crop quality and soil health; in the same line, the delivery of large amounts of fertilizers leads to deterioration in the soil quality and infiltration into water reservoirs causing water pollution (Aktar et al. 2009; Chand Mali et al. 2020; Manjunatha et al. 2018, 2016). On the other hand, nanotechnology studies materials in the range of 1–100 nm related to their properties, manipulation, and production techniques. Nanomaterials have been employed in various applications, including communication, military, food, biotechnology, electrical, chemical, sports, and, most recently, horticulture (Di Sia 2017; Feregrino-Perez et al. 2018; Satalkar et al. 2016). However, the use of nanotechnology in horticulture continues to raise concerns about potential human and environmental consequences. Due to the dimensions of NMs, these materials might bioaccumulate and alter the biochemical systems of human and vegetal processes (Kaphle et al. 2018; Purohit et al. 2017). Nevertheless, the appropriate use of this technology enables the conversion of the traditional agricultural practices into precision agriculture, serving as a tool to diminish the adverse effects of the excesses that the green revolution has left to date and at the same time to enhance plant growth and development (Alvarado et al. 2019).

NMs protect the photosynthetic system and increase photosynthesis, hence increasing plant biomass. Photosynthesis is a biochemical process that occurs in all green plants. It uses photosynthetic pigments, photosystems, electron transport networks, and CO_2 reduction routes to transform light into a chemical form of energy that may be used (Casella et al. 2018). This mechanism has both light and dark reactions which occur in the chloroplast. Salinity, drought, extremes of temperature, a lack of water, floods, high and low light intensity, UV radiation, and other nanoparticles are all stresses that this organelle is vulnerable to (Zia-ur-Rehman et al. 2018). The impact of NMs on photosynthesis is a subject of debate, as some investigations have reported a decrease in growth, chlorophyll content, rates of photosynthesis, leaf stomatal conductance, intercellular CO_2 concentration, and transpiration rates, and inhibition on the expression of genes involved in chlorophyll synthesis and photosystem structure (Wang et al. 2016a; Yan et al. 2020).

Moreover, According to various studies, large quantities of NPs can cause reactive nitrogen species (nitric oxide) and reactive oxygen species (ROS), such as H_2O_2, to be produced reduced nicotinamide adenine dinucleotide phosphate oxidases NADPH

(Tripathi et al. 2017). Accumulation of H_2O_2 is highly toxic for the cell as high levels of this molecule inactivates the photosynthetic mechanism and disturbance of the electron transport system and cellular respiration (Charles and Halliwell 1980; Pandey et al. 2017). However, other studies have proposed several mechanisms of how NMs and NSMs can enhance photosynthesis by increasing the stomatal conductance, the transpiration rate, LHCII (Light-Harvesting Complex II) b gene expression on the thylakoids, photosynthetic assimilation, carboxylation of Rubisco, production of proline and carbonic anhydrase activity, and improving the water splitting and electron transport system (Gao et al. 2006; Pradhan et al. 2013; Siddiqui et al. 2015; Ze et al. 2011). This chapter presents a general review of the interaction of NMs and NSMs with plant tissue, cells, and photosynthesis, considering the beneficial effects these compounds have been implemented to improve crop yield.

4.2 Nanomaterials and Nanostructured Nanomaterials (NMs)

Due to quantum mechanics and surface area properties, NMs have unique physicochemical characteristics that differ from their bulk counterparts. Their new behavior can be applied in different technology areas like sensors, medicine, energy harvest, agriculture, and others (Chavali and Nikolova 2019; Dasgupta et al. 2017; Yang et al. 2019). Their structures are so diverse and have multiple morphologies of a single material that can be synthesized through various methods for obtaining forms with desired geometry, size, crystallinity, tuning, and adjusting their properties to meet the demands depending on the desired application (Sudha et al. 2018).

By definition, nanoscale material dimensions do not exceed 100 nm. However, multiple classifications can be found to categorize these materials depending on their shape, chemical nature, or size, just to name a few (Sudha et al. 2018). Nanomaterials can be classified into four-dimensional groups, 0D, 1D, 2D, and 3D, within the classification by dimensions. Their categorization depends on how many of their spatial dimensions exceed the established range of 1–100 nm (Saleh 2020).

The most straightforward category 0D encompasses those materials whose 3 (depth, length, and height) dimensions are at the nanoscale. In this category, it is possible to find structures such as nanoparticles, quantum dots, nanospheres, or clusters, being the smallest structures, as seen in Fig. 4.1. When one of its dimensions exceeds the barrier of 100 nm, we have designed such as nanotubes, nanorods, and nanowires found in the 1D category; for 2D materials, we can see structures such as thin films, coatings, and nanosheets. Finally, the materials found in category 3D cover structures such as nanocomposites, polycrystals, or MEMs are the most prominent structures (Buzea and Pacheco 2017; Karak 2019; Saleh 2020).

Nanomaterials can also be divided into many classes based on their chemical properties, like carbon nanostructures, organic nanomaterials, metal oxides, quantum dots, semiconductors, and metallic nanomaterials (Patel et al. 2021; Saleh 2020).

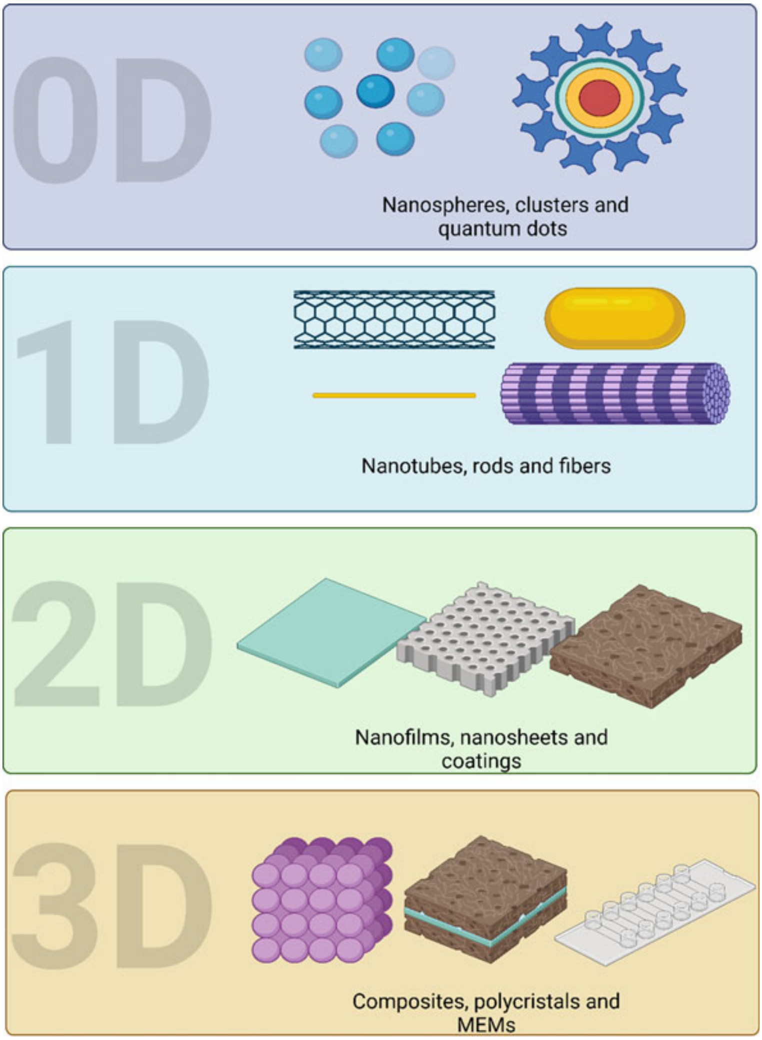

Fig. 4.1 Dimensional classification of nanomaterials (Figure constructed by L. Páramo with BioRender)

4.2.1 Metallic NMs

The most common materials for creating metallic nanostructures are gold, silver, and platinum, which find various applications in the medical area (Carvalho et al. 2019). They can also be made of metallic structures such as aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) (Anu Mary Ealia and Saravanakumar 2017), some of these metallic nanomaterials are applied in catalysis, disinfection, energy storage, and harvest, medicine and more (Schröfel et al. 2014).

Metallic NPs like silver is antimicrobial agents to inhibit or prevent bacterial or fungal diseases in crops (Shah et al. 2021; Vanti et al. 2019) or even sensors for identifying plant disease (Alafeef et al. 2020).

4.2.2 Metal Oxides NMs

Metal oxides are one of the most produced nanostructures worldwide. They are formed by the chemical union of metal and oxygen, such as TiO_2, ZnO, and iron oxides (FeO, Fe_3O_4) (Findik 2021). Some of these materials have semiconductor properties, making them suitable materials for photocatalytic reactions such as titanium dioxide and zinc oxide that are used for photocatalytic water remediation (Saleh 2019), these materials also can eliminate pathogens which promise to be an area of application both in medicine and in the protection of crops against bacteria or fungi that cause diseases (Ziental et al. 2020).

Semiconductor metal oxide like TiO_2 use light for promoting their valence electron through the conduction band; by this procedure, an electron–hole pair is formed, the hole at the valence band acts as an oxidating agent contributing to the formation of $^{\bullet}OH$ radical from the water molecule as seen in Fig. 4.2. The electron at the conduction band functions as a reducing agent where oxygen radicals can be formed. These radicals contribute to the degradation of organic molecules such as pollutants (Chen et al. 2020; Guo et al. 2019). Surface modification by adding other elements or materials can tune the wavelength needed for activating the semiconductors at a wavelength with less energy, making them more affordable and practical (Reddy et al. 2020).

Semiconductors find many agriculture applications, such as fertilizer and growth-promoting agents (Bala et al. 2019; Singh et al. 2019). Semiconductors and other types of NPs can elevate the content of reactive oxygen in plants (Baskar et al. 2018; Javed et al. 2018), which serve as signaling molecules for secondary metabolism and other signaling molecules activation (Marslin et al. 2017). Although an excessive production of ROS could lead to oxidative stress, leading to plant damage, the degree of stress produced by the NPs will depend on their physicochemical characteristics and plant age, species, and type of tissue (Rivero-Montejo et al. 2021).

4.2.3 Carbon-Based NMs

This classification can find multiple phases and structures containing organic carbon bonds. This classification is rich in structures with multiple nanomaterials with different structural and chemical properties (Nasir et al. 2018). In Fig. 4.3, the main structures found in that classification are shown; Fullerenes are ball-shaped structures commonly used as structures for delivering molecules (Kazemzadeh and Mozafari 2019). Graphene is one of the most famous structures among carbon allotropes. It

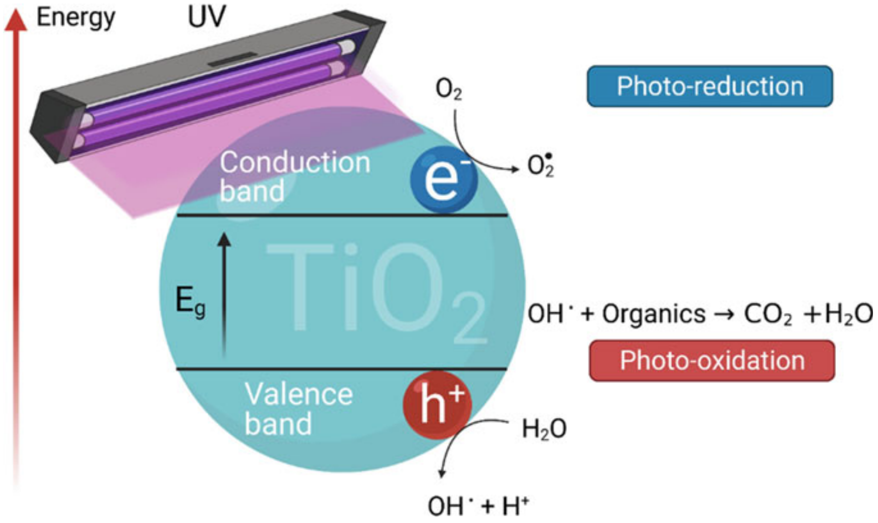

Fig. 4.2 Photocatalytic mechanism of TiO_2 NPs (Figure constructed by L. Páramo with BioRender)

consists of a hexagonal mesh of one atom thick. Because of its structural qualities, this material is well suited to enhance the mechanical properties of materials (Zhong et al. 2017). Another famous structure belonging to this group are carbon nanotubes, which have potential application for creating materials with improved mechanical properties and energy harvesting like solar cells (Batmunkh et al. 2015). Many other structures can be found, such as nanohorns, graphite, and nanodiamonds (Villarreal et al. 2017).

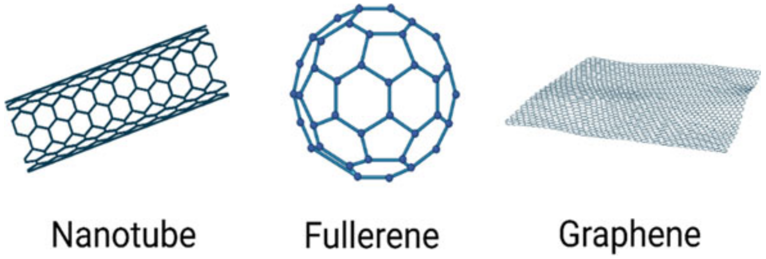

Fig. 4.3 Most used allotropic structures of carbon (Figure constructed by L. Páramo with BioRender)

4.3 Nanomaterials in Agriculture

The use of nanotechnology in the agronomical field can lead to multiple advantages like plant growth improvement, which can be achieved by adding NMs into the media to obtain improved plant features like shoot length, leaf area, root length (Elemike et al. 2019).

The use of nanotechnology in the agronomical field can lead to multiple advantages like plant growth improvement, which can be achieved by adding NMs into the media to obtain improved plant features like shoot length, leaf area, root length (Elemike et al. 2019). The main applications of nanomaterials are shown in Fig. 4.4, showing their uses as materials for improving quality and productivity, nanofertilizers, protection against pathogenic agents or pests, elicitors of plant metabolism, and protecting agents against environmental stresses (Shang et al. 2019; Neme et al. 2021). Aspects such as the improvement in plant growth and an improvement in crop quality can be achieved using nanofertilizers. Their small size allows delivering micronutrients across plant tissues so that the loss of fertilizer is reduced and the positioning of these materials in multiple tissues and organelles is promoted, getting involved in metabolic processes that can favor plant growth (Mittal et al. 2020). On the other hand, protection against biotic and abiotic stresses is achieved by activating genes involved in the defense against stressors due to the interaction and presence of NPs (Khanal et al. 2019). Another highly investigated aspect in the interaction of nanomaterials with plants is the production of secondary metabolites, molecules with various properties and uses such as antioxidants or dyes, the application of nanomaterials has shown in many cases an increase in the production of these compounds through the induction of oxidative stress, which can be used to generate products with a high content of this type of molecules (Marslin et al. 2017).

Nanomaterials show incredible potential to be used as alternatives to modern-day agriculture issues like harmful pesticides, plant sensing, nutrient uptake improvements, stress protection, and many others (Kalita et al. 2021).

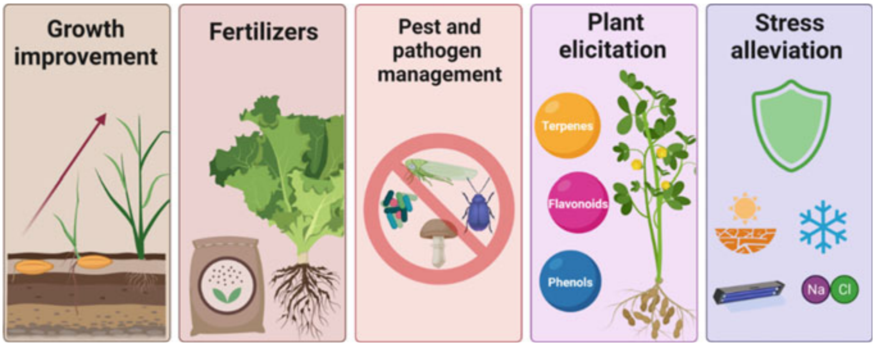

Fig. 4.4 Primary applications of nanotechnology in agriculture (Figure constructed by L. Páramo with BioRender)

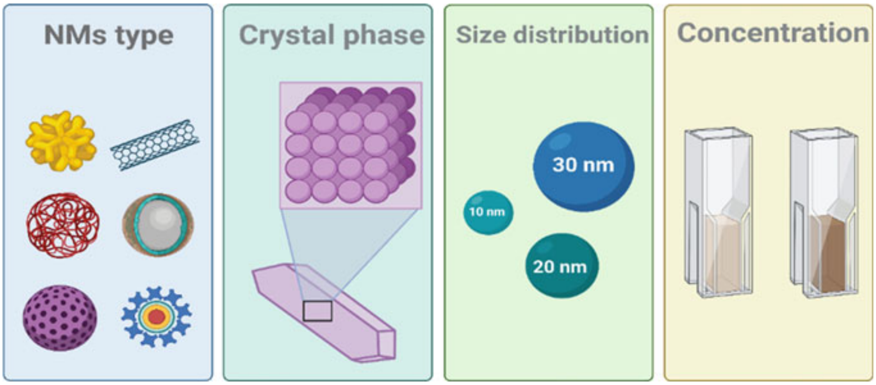

Fig. 4.5 Physicochemical characteristics alter nanomaterials toxicology in plants (Figure constructed by L. Páramo with BioRender)

Nevertheless, care must be taken into understanding plant-NMs interaction. Research in this area finds that NMs with varied physicochemical features can have detrimental effects. Their interactions mechanisms differ from plant species and can affect neighboring organisms that share their ecosystem, like bacteria, fungi, and other plants (Raffi and Husen 2019). Care must be taken into translocation, which is the ability of NMs to travel through organs and distribute in different areas. Their translocation into higher organs can be significant in movement through the food chain (Tripathi et al. 2017).

Toxicology and interaction mechanisms are very complex since these phenomena depend on the physicochemical characteristics of the nanoparticle. Between the most important features affecting NMs-plant interactions, we find the size, crystal phase, agglomeration, morphology, time, and dose of exposure, among others, as shown in Fig. 4.5 (Zia-ur-Rehman et al. 2018).

NMs in agriculture can bring some clever alternatives for improving crop quality and durability. New formulations combined with a secure and conscious understanding of NMs' fate and effect on the environment can provide safe and economical products for crop development upgrade.

4.4 Interaction of NMs and NSMs with Plant Tissue and Cells

Plants constantly interact with biotic and abiotic factors during the whole day, either at daylight or night. Regarding the stimulus, plants usually orchestrate a specific and highly regulated response. The triggers are perceived by different plant tissues such as leaves, stems, roots, and even at the cellular level if we consider the stomata or the cuticle present in the leaves or stems. The nanoparticles (NPs) and the nanostructured materials (NMs) are considered abiotic elements present in the environment with different origins and interact with the plant's tissues. Naturally, plants have other cellular structures and tissues, all capable of interacting with NPs and NMs. Once the plant has absorbed these compounds, they usually are stored or interfere with some cellular mechanisms. Considering the plant at the anatomical level, these materials can interact with pollen grains, leaf cuticle, stomata, the vascular system consisting of xylem and phloem, and root hairs. To get into the plant's cells, the NPs and NMs use several mechanisms like clathrin-mediated endocytosis (Chichiriccò and Poma 2015; Tripathi et al. 2017). Once inside the plant, these materials are disseminated through the xylem and phloem, reaching several plants' tissues considered a systemic transport (Tripathi et al. 2017).

For the NPs to interact with the cells in the plant and with deeper tissues, the translocation of these materials must be carried out, which is related to several factors, like the zeta potential, which is the intensity of the electric field of a double layer of charges, which in this case would be the charges that are outside the plant cell and the charges of the molecules with which contact is being established. Those NPs positively charged (20–30 Mv) are internalized through the plant's cell and more accessible by the chloroplast than NPs whose net charge is neutral (Hu et al. 2020). If the size of the NPs decreases, the zeta value that needs to be translocated is higher. Related to the size and charge, using carbon dots as a model, CeO_2(NC) and SiO_2 changes in the translocation through the leaves were observed in cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.). By confocal microscopy was observed that NPs around 16, 8, and 2–6 nm could cross the stomata in epidermal cells by no endocytic pathway. These particles can travel the lipid layer. Most NPs with a 16 and 8 nm hydrodynamic size does not pass the guard cells' layer on the leaves. In contrast, small NPs around de 6–8 nm positively charged and 2–6 nm negatively charged are transported to the extracellular space easier. The translocation process is related to the zeta potential and the size of the material to be transported inside the plant tissues (Hu et al. 2020).

In addition, it is essential to consider is the chemical characteristics of the plant cellular surface. The cell wall and the membranes have many organic and inorganic molecules that produce pH changes, modify the ionic interactions and show different oxidative potential in the extracellular milieu. Altogether, these traits resulted in a dynamic charge equilibrium in the extracellular milieu due to the charges presented in the cell wall and the membrane whose net charge is negative and the anchored proteins. Several NPs are positively charged, then the interaction with the plant cells is straightforward. It is worth mentioning that these interactions also depend on environmental conditions, such as the value of the pH of the soil. On the other hand, there are NPs with a negative net charge. Usually, these materials are covered with positively charged molecules to improve their capacity to interact with the plant (Juárez-Maldonado et al. 2019).

Once the NPs or CNEs interact with the plant, these are systematically distributed through the xylem and phloem. In corn plants and *Arabidopsis thaliana* L., the vascular system transports the Cu_2O, acetate-Cu, and $Cu_2(OH)PO_4$ NPs from the roots to the leaves and even can reach the flowers (Wang et al. 2016b). The clathrin-endocytosis of gold NPs was investigated in tobacco cell cultures. The formation of vesicles was observed by transmission electron microscopy (TEM), which travels until the late endosomal. Interestingly, the traffic was drastically diminished in the treatment with IKA, an inhibitor of clathrin synthesis.

Nevertheless, the NPs were internalized by other routes (Onelli et al. 2008). The independent clathrin mechanism was also observed in cell cultures of *Vitus vinifera* L., using NPs of poly(lactic-co-glycolic) acid even when the culture is treated with the IKA inhibitor wortmannin and salicylic acid (Palocci et al. 2017). A similar phenomenon was observed using multiwalled carbon nanotubes transported by endosomes and chloroplasts in *Catharanthus roseus* L. Interestingly; these endosomes travel to Goldi to other organelles like mitochondrion, chloroplast, and nuclei (Serag et al. 2011).

Another essential factor to consider related with the NPs and their interaction with plant tissues is the plant species involved, since there are physiological and anatomical differences, to mention some: the mesophile cells on corn leaves, being the extracellular area reduced, then the possibility to establish contact between the NP and the cells is minor, whereas, in cotton (*Gossypium hirsutum* L.), the amount of stomata is higher, then facilitating the entrance of NPs where the permeability is notably increased (Hu et al. 2020).

Similar behavior was observed in *Zea mays* L. and soja (*Glycine max* L.), where the NPs, after internalized, agglomerate and move between organelles. Similar behavior was observed with multiwalled carbon nanotubes, which are smaller and positively charged due to the presence of amino groups (Serag et al. 2011; Zhai et al. 2015). Inside the cell, the NMS also can adhere either in the plant tissue or specifically in the cells. The degree of adhesion depends on the chemical compounds present in the plant species. In *Arabidopsis thaliana* L. seeds, TiO_2 and TiO_2-Cu^{2+} particles adhere on the surface, causing no germination or nutrient absorption effects. Also, the accumulation of gold or copper resented in Ag NPs, or TiO_2-Cu^{2+} was observed after the seed germination (Garcidueñas-Piña et al. 2016). On the other hand, in tobacco roots, the absorption of silver was increased after the exposition to Ag NPs or $AgNO_3$, which was accumulated in the root tissue and lesser amount on leaves (Cvjetko et al. 2018). The accumulation of NPs produced with CuO in lettuce (*Lactuca sativa* L.), and cabbage (*Brassica oleracea* L.) was detected in leaves and roots (Xiong et al. 2017).

Magnetic NPs 12.5 nm in size produced with FeO can penetrate the roots in *Cucurbita pepo* L. but do not translocate to leaves or flowers since they remain accumulated on the roots' cells. Interestingly, its accumulation due to its magnetic properties was detected in the plasmodesmata and stomata, disturbing the cell-to-cell communication transport of nutrients (Tombuloglu et al. 2019). When chelating agents like EDTA were added, iron in the roots increased at a 250 mg/kg concentration. Whereas the supply of magnetic NPs without any other molecule, only 10.37 mg/kg of iron was detected, suggesting that the presence of cargo molecules is essential to directly modify the absorption and accumulation of iron NPs (Tombuloglu et al. 2019).

NMs, on the other hand, have been demonstrated to hinder plant growth and development. NPs of TiO_2 and TiO_2-Cu^{2+}, reduces the plant growth (Garcidueñas-Piña et al. 2016). In *Lactuca sativa* L., the biomass was reduced by 250 mg/mL. In contrast, the biomass was increased at 1 mg/mL (Xiong et al. 2017). When beans plants (*Vigna vulgaris* L.) were exposed to ZnO and TiO_2, the seed germination rate was higher at a concentration of 20 to 100 μg/mL. Also, the size of the sprouts was increased when the concentration of NPs was higher. Interestingly, the size of the roots was raised at a concentration of 20 μg/mL, which decreased by the increased application of material, showing an apparent dose–response effect (Jahan et al. 2018).

In contrast, in the case of turnip, the treatment with Ag NPs at 1 mg/L negatively affects the growth of sprouts. Also, the rate of germination was reduced to 31%. In contrast, the root's size at a low dose (1 mg/L) 1 cm increment was observed; contrary, if the Ag NPs increased to 5 mg/L, the growth of the roots was drastically reduced (Thiruvengadam et al. 2015). Interestingly, adding other molecules together with NPs o CNEs like Fe-NPs shows a different effect. The addition of Fe-NPs reduces the plant's biomass, but EDTA reverted the adverse impact and increased biomass production (Tombuloglu et al. 2019). At a concentration of 1000 mg/mL of NiO, the seed hardly germinates, whereas with other NPs, the size of the plant diminishes when the concentration is increased from 100 to 1,000 mg/mL of CuO, reducing the size of the root. Also, a reduced growth was observed due to the treatment with ZnO. This effect was attributed to oxidative damage that affects the size of the root. In the case of *Solanum melongena* L., the treatment with NPs of NiO at concentrations of 1000 mg/mL a delay in germination was scored, whereas with NPs ZnO and CuO, the size of the plants was reduced related to the amount of material applied, from 100 to 1,000 mg/mL. Similarly, the size of the root of *Solanum melogena* L. was also reduced after the treatment NPs of CuO (Baskar et al. 2018).

However, nanostructured biosilica can adhere to two cell types present in rice leaves (*Oryza sativa* L.), which differ in the kind of constructs produced after exposure. The long silicified cells are silica plates and the buliform fan cells (Sato et al. 2017). Interestingly, this biosilica is formed from absorbed silicates from soil which, after its accumulation, cover the leaves with a weak layer around 1 μm depth. This phenomenon provides hardness to the leaves. Then it could be implemented in plants protection to avoid viral infections, herbivory, or support against mechanical stress (Sato et al. 2017). In tobacco, many vacuoles were observed in the cells of the roots at 100 μM concentration of NPs covered with citrate. Also, elongated vacuoles were observed in the nucleus, with adverse effects capable of destroying the whole-cell starting from the nuclei (Cvjetko et al. 2018).

Once the NMs are inside the cells induce oxidative stress in the plant, this is highly related to the genetic expression of protective antioxidants mechanisms (Baskar et al. 2018). In *Arabidopsis thaliana* L., the number of anthocyanins increases at 2.5 mg/mL of nanostructured $AgTiO_2$. This molecule relieves oxidative stress. Then it is increment because the plant is struggling with this kind of stress (Garcidueñas-Piña et al. 2016). In eggplant (*Solanum melongena* L.), the NPs of NiO, CuO, and ZnO showed a negative effect on the chlorophyll synthesis, also the production of anthocyanins, phenolic compounds, and flavonoids was reduced, as a consequence, a high amount of ROS such as H_2O_2, OH^- and O_2 were scored (Baskar et al. 2018). Due to uncontrolled ROS generation, lipid peroxidation was observed; also, the amount of malondialdehyde (MDA) was higher, which is a lipid oxidation biomarker. In corn, Ag NPs covered with citrate in root tissue increases the presence of MDA. Also, the antioxidant enzyme superoxide dismutase (SOD) was detected in the leaves but not in the roots, suggesting a differential gene expression (Cvjetko et al. 2018).

Similarly, lipid peroxidation was scored, a concomitant reduction in the synthesis reduction of chlorophyll anthocyanins. Contrary the ROS production was increased. In response to this oxidative stress, the synthesis of carotenoids, phenolic compounds, and glucosinolates was increased (Thiruvengadam et al. 2015). The treatment of *Vigna angularis* L. with NPs of ZnO and TiO_2 under a hydroponic treatment triggers the activity of glutathione reductase, superoxide dismutase, and ascorbate peroxidase (Jahan et al. 2018).

Plants' responses to oxidative stress are influenced not just by changes in molecule concentrations and antioxidant enzyme activity but also by the amount of chlorophyll present, which is associated with the damage in chloroplasts. In *Solanum melogena* L., the treatment with CuO-NPs reduces the amount of chlorophyll when the concentration of NPs increases. The chlorophyll was decreased to 3.5 mg/g after 1000 mg/mL CuO (Baskar et al. 2018). In *Vigna angularis* L., the number of chloroplasts was reduced due to ZnO and TiO_2 related to oxidative stress (Jahan et al. 2018). Even in some studies was demonstrated that NPs produce oxidative stress.

In some cases, they mitigate it and are beneficial, like Fe NPs in wheat (*Triticum vulgare* L.) at 0.1 M they induced oxidative stress. However, at 0.025 M NPs concentration, a beneficial effect was observed (Korotkova et al. 2017). Since some NPs agglomerate, the ions are slowly released, showing lesser cytotoxicity allowing the plant to orchestrate a proper response to that specific stimulus. Nevertheless, if the high concentration of NPs exceeds the antioxidant capacity supplied, a cytotoxic effect is observed (Korotkova et al. 2017). This duality was also studied in *Arabidopsis thaliana* L. treated with 1 mg/mL of Ag-TiO_2. The amount of chlorophyll was increased to 1.5 mg/g, contrary to 2.5 mg/mL of Ag-TiO_2, reduced to 0.7 mg/g (Garcidueñas-Piña et al. 2016).

4.5 NMs and NSMs and Photosynthesis

From the essential photophysics of light absorption and excitation energy transfer through the gas exchange in leaf and canopy, decades of research have yielded a complete understanding of photosynthesis. Furthermore, recent developments in computer power have been crucial for better understanding photosynthetic processes and predicting the results of various redesigns of this biological system (Ort et al. 2015). As a result, photosynthesis is a biological process in which the sun's energy is caught and stored through a series of actions that turn the pure energy of light into the free energy required to sustain life. Furthermore, ideal conditions for photosynthesis are necessary, including a vast supply of water and nutrients to the plants and optimal temperature and light conditions (Adams et al. 2020; Shweta et al. 2016).

In this way, it has been established that plants do not reach their maximal photosynthetic efficiency. This determinant is not close to its biological limits. It, therefore, has become a key motivator for researchers that have proved several tools and technologies, such as nuclear transformation, plastid transformation, mitochondrial transformation, multigene engineering, protein design, synthetic genomics, phenotyping in the field, nanotechnology, development of sensors for light intensity, quality, temperature, CO_2 concentration, air humidity, and relative humidity (Ort et al. 2015; Swift et al. 2019). Plants' ability to collect and utilize solar energy for the photochemistry involved in this biological process is frequently limited. As a result, employing nanomaterials as synthetic probes to improve how plants use light is a hot issue for new research. The premise is simple: plants' capacity using sunlight or artificial light can be optimized with nanomaterials and nanostructures (Swift et al. 2019). Enhancing the capacity of plants to interact with and optimize the capture of light can increase the productivity of crops and enable the tailoring of plants for different environments where there is an exceeding of solar radiation or light might be a limited resource. Since nanomaterials' synthesis and surface modification have overcome previous drawbacks, this technology has been raised as potential probes to enhance photosynthesis and, therefore, crop production through the increase in biomass (Swift et al. 2019).

Under prevalent ambient conditions, the amount of light is not optimal for photosynthesis. The intensity of sunlight is substantially higher than that required for photosynthetic saturation, resulting in dissipation and loss of the surplus absorbed energy as heat or fluorescence (Melis 2009). The highest observed photosynthetic efficiencies are 30% lower than theoretical efficiency due to photosynthesis's light saturation. The photosynthesis light saturation curve describes net photosynthesis as oxygen evolution as a function of light intensity, defining a non-linear curve as insolation increases, with a maximum value where the curve flats, known as P_{max}. In C3 leaves, photosynthesis is saturated by approximately 25% of the maximum sunlight (Melis 2009).

Furthermore, light intercepted above this amount will lower photosynthetic efficiency in proportion to the excess light absorbed. Therefore, brighter sunlight does not translate into greater productivity by the cells (Ort et al. 2011). For example, in a day with a profile of daily solar photosynthetically active radiation at sea level, where more than 2000 mmol photons m^2s^{-1} are reached at 12 h, more than 80% of absorbed solar irradiance would be wasted in the photosynthetic apparatus, saturating at about 7 a.m. and remain saturated until 5 p.m. (Melis 2009). This phenomenon has been considered in the photosynthesis model. In this way, earlier computational models incorporated only light as an environmental variable. However, novel models include CO_2 concentration, temperature, air humidity, leaf physiology, leaf nitrogen content, and leaf canopies characterized by vertical gradients in leaf photosynthetic properties. Considering that the saturation of the photosynthetic apparatus applies to cells and chloroplasts directly facing the sun in the outer part of the canopy while leaves inside the canopy might be exposed to low amounts of light (Hikosaka et al. 2016; Melis 2009; Ort et al. 2011).

Melis (2009) discovered that the carbon processes are responsible for the rate restrictions in photosynthesis (slow rate of catalysis by Rubisco). The thylakoid membrane's Cytochrome b6–f complex acts as a bottleneck for electron transport and the relatively slow turnover rate of the Mn-containing H_2O-oxidation complex. Hence, chloroplast and cellular metabolism for biomass generation entail energy loss, lowering the solar energy conversion efficiency. From the 100% of the incident sunlight energy, 30% is stored as chemical energy in the photosystems. Only 8% to 10% is converted into biomass.

Long et al. (2006) proposed six possible mechanisms that might increase the solar radiation conversion efficiency that may be achieved by providing the material that could be introduced into plant breeding programs: (1) Rubisco with decreased oxygenase activity, but without decreased catalytic rate, (2) Efficient C4 photosynthesis engineered into C3 crops, (3) Improved canopy architecture, (4) Increased rate of recovery from photoprotection of photosynthesis, (5) Introduction of higher catalytic rate foreign forms of Rubisco, and (6) Increased capacity for regeneration of RuBP via overexpression of SbPase.

Other essential plant mechanisms that affect plant photosynthesis are related to some organs or specialized cells involved in the gas exchange with the environment. The size of some NPs or NMs makes them possible to allocate through the plants' structural components and vascular systems. Moreover, the length of cellular organelles or elements in the membranes is equivalent to the size of some NPs. This characteristic allows specific permeability that induces genomic and metabolic changes, widely studied as adverse biological effects. Nevertheless, depending on the material and dose, the exposure might cause beneficial effects on general plant performance, growth, and photosynthesis (Aguirre-Becerra et al. 2021; Shang et al. 2014; Vecchio et al. 2012). For instance, plants have specialized guard cells called stomata, which are present in the upper and lower surface of the epidermis of leaves. They act as turgor-operated valves that limit water loss and CO_2 intake, a helpful adaptation for photosynthesis and other stressful circumstances. They are relatively small and considerably variated in size between species (Camargo and Marenco 2011; Naeem et al. 2019).

The dimensions of the outer limits of their cell walls may vary according to the stomatal aperture, from under 10 to almost 80 μm in length and from a few micrometers to about 50 μm in width (Camargo and Marenco 2011; Willmer and Fricker 1996). Similarly, bordered pits and cavities in the lignified cell walls of xylem conduits are essential in higher plant water transport systems and help to prevent embolism and vascular infections from spreading into the xylem. They have a maximum pit membrane porosity of 5–420 nm in most angiosperm species, although it should be stressed that these values are usually <100 nm (Choat et al. 2006, 2008; Shane et al. 2000).

Moreover, at a cellular level, pores of the primary cell wall are pathways for penetration of molecules into the cell, consisting of a polysaccharide-proteic structure with a size ranging from 3.5 to 20 nm (Carpita et al. 1979; Chichiriccò and Poma

2015; Fleischer et al. 1999). In the same way, the transport of molecules from cell to cell is possible through plasmodesmata, cytoplasmic channels with 20–50 nm in diameter, which usually let the pass of particles, around 3 nm (Chichiriccò and Poma 2015; Dietz and Herth 2011).

The import of proteins into chloroplasts is a need for photosynthesis. Where protein translocation of the outer and inner chloroplast membranes, which are responsible for the import of about 95% of all chloroplast proteins from the cytoplasm, makes this process possible (Shi and Theg 2010; Shi et al. 2013), and they are considered to have a pore size greater than 2.56 nm (Ganesan et al. 2018; Huang et al. 2010). In this way, the deep knowledge of photosynthesis, at a biochemical level and with the implementation of computational modeling, and the plenum understanding of plant physiology, makes nanotechnology a feasible tool for achieving the exposed mechanisms for optimizing this biological process, and therefore, increase crop yield; some examples are disclosed and discussed in the following paragraphs.

Cao et al. (2017) investigated the effects of cerium oxide nanoparticles (CeO_2 NPs) with two different surface characteristics (uncoated and polyvinylpyrrolidone (PVP)-coated) on soybean photosynthesis at four different doses (0, 10, 100, and 500 mg/kg dry soil. They discovered that the physiological effects of NPs are dependent on their concentration and surface coating qualities, with bare CeO_2 NPs showing a 54% increase in photosynthesis rate and PVP-CeO_2 NPs showing a 36% increase in photosynthesis rate at the concentration of 100 mg/kg. Moreover, the maximum rate of Rubisco carboxylase activity increased by 32% and 27% for bare and PVP-coated CeO_2 NPs, respectively, at the same concentration. In addition, PVP-CeO_2 NPs significantly increased the total dry weight (24% and 45%), total dry root (89% and 95%), and dry shoot biomass (15% and 28%) at 10 and 100 mg/kg, respectively.

However, at 500 mg/kg, the net photosynthetic rate was lowered by roughly 36% for both nanoparticles. Rubisco activity was suppressed, and CO_2 diffusion pathways were hampered at concentrations more than 500 mg/kg; Furthermore, the overall chlorophyll concentration of the treatments did not differ much. This experiment shows that soybean plants exhibited significantly higher P_{max} when treated with CeO_2 NPs at 100 mg/kg, a convenient characteristic at high light intensities.

Another example is the experiment of Faizan et al. (2018) where the effect on growth and photosynthetic efficiency of tomato plants was evaluated; with an experimental design consisting on plants with a 20-d stage of development which roots were dipped into 0, 2, 4, 8, or 16 mg/L of zinc oxide NPs (ZnO NPs) for 15, 30, and 45 min and then grown under natural environmental conditions, resulting in an increase in the chlorophyll content in all treatments under different durations of exposure, where the concentration of 8 mg/L (ZnO NPs) dipped for 30 min before transplantation showed a maximum value of this variable over all the other treatments and durations; furthermore, this treatment presented the highest values of net photosynthetic rate (50.7%), stomatal conductance (34.4%), intracellular CO_2 conductance (27.9%), transpiration rate (32.0%), protein content (45.0%), shoot length (35.8%), root length (28.6%), shoot fresh matter and dried matter (21.9 and 27.6%, respectively), and leaf area (27.9%), and a considerable increase in antioxidant capacity of CAT (69.7%), POX (65.0%), and SOD (80%) in comparison with their control plants, concluding that the exposition with this nanoparticle generates a promising response in tomato plants as the growth and photosynthetic efficiency were increased.

In the same line, Li et al. (2015) evaluated the effect of a wide range (0, 500, 2500, 4000 mg/L) of nano-TiO_2 in plants of oilseed rape (*Brassica napus* L). They found that chlorophyll content was significantly higher and followed a nano-TiO_2 concentration-dependent pattern, with the highest chlorophyll content in plants treated with 4000 mg/L nano-TiO_2, resulting in increased root length, plant height, and fresh biomass. Furthermore, photosynthetic parameters like net photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate all increased significantly over the next few weeks, with maximum performance at the end of the experiment with seedling treated with 4000 mg/L nano-TiO_2 concentration except for intercellular CO_2 concentration. There were no significant chloroplast ultrastructural changes, and transmission electron microscopic (TEM) images revealed intact and typical grana and stroma thylakoid membranes in the chloroplast, indicating that nano-TiO_2 did not induce the stressful environment within the chloroplast, implying that nano-TiO_2 has growth-promoting effects on oilseed rape plants without phytotoxic effects.

In the case of Yoon et al. (2019), The phytotoxic effects of nanoscale zerovalent iron (nZVI) on photosynthesis and related metabolic adaptability of soil-grown *Arabidopsis thaliana* L. oilseed rape plants were examined, increasing plant biomass (38%) and accumulation of carbohydrates: glucose (44%), sucrose (27%) and starch (52%) due to the enhanced photosynthesis, which was confirmed by the increase in the CO_2 assimilation rate (26%), stomatal conductance (40%), and transpiration rate (47.61%).

In the research of Li et al. (2020), Fe and Fe_3O_4 (magnetite), both naturally occurring nanosized crystals and minerals, were foliar sprayed to 4-week-old maize (*Zea mays* L.) plants for ten days, with results showing that Fe NPs boosted maize leaf net photosynthetic rate and chlorophyll content by 19.9% and 19.3%, respectively. Furthermore, Fe_3O_4 NPs for 27.5% and 26.1%, respectively, leading to a significant increment in plant biomass by Fe and Fe_3O_4 NPs by 31.8% and 34.6%, respectively, concluding that the trade-off of energy-consuming pathways might be an alternative explanation for the enhanced photosynthesis and that the use of this nanoparticle has the promising potential to be used in nano-enabled agriculture to promote plant growth.

An interaction of carbon-based nanomaterials with chlorophyll-a, chlorophyll-b, and accessory pigments in the chloroplasts has been proved to modify the ability of plants to harvest light energy. Therefore, this technology works as artificial antennae allowing chloroplasts to take advantage of wavelengths that are not considered to be essential for photosynthesis, such as green and near infra-red, considering that red, blue, far-red, and UV-A are the wavelengths that most plants use for this process (Aguirre-Becerra et al. 2020; Cossins 2014; Giraldo et al. 2014; Siddiqui et al. 2015).

González-García et al. (2019) evaluated the effect of CNTs and graphene applied via foliar or drench in tomato resulting in an increase of 87% in root biomass, 72% in chlorophyll-a, and 39% in chlorophyll-b. Moreover, Younes et al. (2019) studied the effect of graphene nanosheets on pepper (*Capsicum annuum* L.) and eggplant

(*Solanum melongena* L.) in two years of cultivation, finding an increase of 80% and 81% in fruit yield, 144% in chlorophyll-a, 132% in chlorophyll-b, and 143% in carotenoids for eggplant. And an increase of 121% and 119% in fruit yield, 126% in chlorophyll-a, 134% in chlorophyll-b, and 42% in carotenoids for pepper, for years 1 and 2, respectively.

Carbon dots (CDs) are a new member of fluorescent carbon material with a diameter below 10 nm. They are becoming a promising alternative for their application in living organisms due to their composition and biocompatibility (Tuerhong et al. 2017). Wang et al. (2018) described their effect on mung bean sprouts (*Vigna radiata*), reporting an increase in the electron transportation rate of the photosystem 1 (PSI) of leaves (8.8%), chlorophyll content (14.8%), and general photosystem activity (10.4%). Li et al. (2018) fabricated a series of CDs of ~5 nm with different oxygen contents that were employed as a model material to explore the impact of these molecules on rice, finding that CDs can penetrate all parts of the plant, including the cell nucleus, loosening the DNA structure, and inducing the thionine (Os06g32600) gene expression; moreover, CDs promoted an increase of seed germination, root elongation, seedling length, RuBisCO activity, and carbohydrate generation. The plant can degrade cDs to form plant hormone analogous that promote growth and CO_2 that is converted into carbohydrates through the Calvin cycle of photosynthesis.

The RuBisCO activity was significantly increased by 42%, indicating that CDs can improve the transformation efficiency of photosynthetic products. Furthermore, Park and Ahn (2016) evaluated the effect of multiwalled CNTs (up to 2000 mg/L final concentration) in carrots (*Daucus carota* L.). In the seedling process, a significant increase in the total chlorophyll content of the leaf tissue was found by 25–30%, but the seedling growth decreased 10% in the same species.

Siddiqui et al. (2019) evaluated the effect of foliar spray of graphene oxide, reporting a significant increase in total chlorophyll (27%) and carotenoids (18%). In contrast, Zhang et al. (2016) evaluated different doses of graphene in roots and shoots of wheat (*Triticum aestivum* L.) plants, reporting an inhibition of long-term biomass production as the chlorophyll content and PSII (photosystem II) activity were reduced. Moreover, Vochita et al. (2019) studied the effects of graphene oxide in seedlings of the same species, reporting a decrease of 22.48% in chlorophyll-a.

4.6 Conclusion and Prospects

The agriculture sector is the backbone for feeding humans globally. Feeding the continuously growing human population is a significant challenge to researchers in the modern era. Therefore, developing new innovative technologies to enhance production in modern agriculture are cost-effective and eco-friendly for sustainable development globally. The unique properties of NMs and NSMs make them a viable choice for the design and development of sustainable horticulture; however, in this field, the use of nanomaterials is relatively new and needs further exploration (Feregrino-Perez et al. 2018). Because these compounds can potentially affect the biochemical mechanisms of human and vegetal processes, the field of nanotoxicology was formed to investigate the toxicological and environmental effects, physicochemical and hazardous properties, biological mechanisms, and conditions of nanoparticles used in horticulture and the entire food chain (Alvarado et al. 2019; Feregrino-Perez et al. 2018; Kaphle et al. 2018; Li et al. 2015).

Photosynthesis is essential in every green plant since it is related to the primary metabolism and, therefore, is responsible for generating the plant biomass, inducing its growth and development. Several NMs and NSMs have been proved to improve the diverse mechanisms of this biochemical process, from an enhancement in the capture of sunlight by the photosynthetic pigments and photosystems, a more efficient electron transport chain and CO_2 reduction pathways, increment in the stomatal conductance, photosynthetic assimilation, carboxylation of Rubisco, production of proline and carbonic anhydrase activity, water splitting, among others. More research must be performed to assure that NMs and NSMs are safe to produce food. Several variables must be considered, such as the type of nanomaterial, dose, bioaccumulation, and possible risks to human health and the environment.

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Chapter 5
Interactions of Nanomaterials with Plant Pigments

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Abstract The applications of engineered nanomaterials continue to expand into agri-food production, particularly in relation to nano-enabled agriculture formulations and/or colloidal particles. The widespread use of nanomaterials has generated concerns given the impact these nanostructures could be having on biomolecules,

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cellular homeostasis, and internal compounds. Understanding the interaction mechanisms between engineered nanomaterials and plant pigments is essential for unbiased assessments of their internalization, trafficking, behavior, and fate into cellular structures through a molecular lens. This chapter describes the mechanisms that drive these interactions on the cellular uptake and trafficking of nanomaterials. Furthermore, we discuss different examples of how plant photosynthetic and non-photosynthetic pigments can helpful to understand the behavior of nanomaterials in distinct plant tissues. Finally, we demonstrate some experimental (in vitro) and theoretical (in silico) methods aimed at monitoring and understanding existing molecular interactions of nanomaterials with plant pigments.

Keywords Engineered nanomaterials · Molecular interaction · Pigments · Plant systems · Homeostasis · Nanotoxicity · Environmental chemistry

5.1 Introduction

The growth of the nanotechnology sector has raised concerns about its environmental impacts. The release of nanomaterials (NMs) into the environment may induce potential adverse effects on natural ecosystems. In particular, terrestrial plants, which are the primary producers of the global food chain, are severely affected by NMs, raising concerns about this theme. Nanotechnology has shown great potential for addressing productivity problems in the agri-food sector (White and Gardea-Torresdey 2018; Bartolucci et al. 2022; Kandhol et al. 2022). Consequently, nano-enabled plant protection products can play an important role in the future of agriculture (Grillo et al. 2021; Pontes et al. 2021). Understanding the interaction mechanisms of NMs with plant pigments is crucial for unraveling their potential side effects on the metabolism, biochemistry, and physiology of these organisms when NMs are internalized. Negative cellular feedback related to nanostructured materials interactions with pigments can trigger complex allosteric adjustment responses or impair their metabolic pathway when extrapolating the homeostatic normal range (Santiago et al. 2020). The adjustment of cellular responses is related to their homeostatic ability. Thus, any structural or functional changes in plant pigments, particularly photosynthetic ones, impact the photosynthetic performance and biomass productivity (Tighe-Niera et al. 2018). Such nanomaterial–pigment interactions may represent a novel opportunity to increase crop production.

Plant organisms can produce more than 200,000 different types of chemical compounds (Fiehn 2002; Čepulytė and Būda 2022). Colored compounds are a special class, called "pigments," and include flavonoids/anthocyanins, betalains, carotenoids,

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and chlorophylls (Mulder-Krieger and Verpoorte 1994). The interaction of nanoarchitectured materials with plant pigments has been studied in the last decades, with important advances in several areas such as industry, fiber dying, medicine, pharmacology, food, and others (Shaid-ul-Islam and Mohamad 2013; Venil et al. 2020; Yu et al. 2021). Although, nanoparticle–pigment interaction has been explored and exploited extensively, few reports have systematically presented the mechanisms involved such as the factors affecting this interaction and their effects on plant fitness. Nanoparticles (NPs) have been implemented in agricultural systems as nanofertilizers and nanopesticides for crop growth regulation and disease control, respectively (White and Gardea-Torresdey 2018; Grillo et al. 2021; Lima et al. 2022). Consequently, due to their uptake and internalization, their interaction with biological molecules onto cellular media is expected.

Natural organic pigments are colored compounds produced by some organisms with a wide range of biological finality, i.e., communication, protection, reproduction, and others. In plants, pigments play a key role in light absorption for chemical energy production due to their physical properties of light absorption, trapping, and transport in the visible region of the electromagnetic spectrum. Depending on their chemical structure, pigments are responsible for a great variety of colors and fragrances of flowers, fruits, seeds, and foliage (Mulder-Krieger and Verpoorte 1994).

Molecular dynamics involved in the interaction of plant pigments with NPs have been successfully studied by biophysical, biochemical, biomolecular, and electrochemical behavior (Barazzouk et al. 2012; Mezacasa et al. 2020; Pontes et al. 2020; Bhogaita and Devaprakasam 2021). In general, these interactions were evaluated to understand: (*i*) the environmental implications of NMs, (*ii*) the physiological responses and biological phenomena, and (*iii*) to improve artificial photosynthesis and solar cells.

In this chapter, we discuss available data concerning engineered nanomaterials (ENMs) and their interaction with plant pigments in their biological properties. Contradictory results have been reported about the effects of NMs on different plant pigments. Detection techniques, possible in vivo implications, and side effects are discussed and an analysis of future research needs is also included.

5.2 Nanomaterial's Cellular Uptake and Intracellular Transport

Engineered nanomaterials are emerging as delivery vehicles for biomolecules in plants, especially for plant genetic transformation (Zhao et al. 2018), biostimulants (Juárez-Maldonado et al. 2019), fertilizers (Kopittke et al. 2019), and pesticides (Pontes et al. 2021). However, the incorporation and target of NMs in plants are challenging due to the presence of plant cell walls, differences in membrane chemical compositions compared to mammals' cells, and low endocytic rate (Miralles et al. 2012; Husen and Siddiqi 2014).

The first barrier that ENMs must overcome to entry the cell is the cell wall. Regardless of the organ of the plant (in leaf epidermis, for example, there is also a cuticle, a layer that covers the outer periclinal walls and is composed mainly of lipid substances).

Plant cell walls are mainly composed of cellulose, hemicelluloses and pectins, and a large number of proteins are involved in the cell dynamics through diverse functions such as environmental sensing, growth, signaling and defense (Fry 2004; Hijazi et al. 2014). For a long time, the cell wall was taken as a non-dynamic and functional limited structure, however, this view has changed drastically.

Some authors argue that the movement of molecules through the wall is limited by the pore size. The pore size is defined as the space between the cell wall components and the wall matrix (Salmén 2004; Rondeau-Mouro et al. 2008; Kurczynska et al. 2021). The cell wall pore size of several species has been documented e.g. *Chenopodium album* Linn. (Chenopodiaceae) in which the pore sizes vary from 3.3 to 6.2 nm, depending on experimental conditions (Fleischer et al. 1999). In *Achlya bisexualis* Coker & Couch (Saprolegniaceae) the pores in the wall were determined to be 2–3 nm (Money 1990). In *Hordeum vulgare* Linn. (Poaceae) roots the pore size of rhizodermal cell walls was 3.2–3.8 nm (Milewska-Hendel et al. 2017). This hypothesis seems unsupported and unlikely since much larger sized NPs were noticed within plant tissues, even when they contacted the outer wall. *Glycine max* Linn. merrill (Fabaceae) and *Medicago sativa* Linn. (Fabaceae) plants absorbed citric acid-coated magnetite Fe_3O_4NPs about 18 nm diameter. These particles accumulated in roots. Little or no translocation to the aerial parts was observed (Iannone et al. 2021). *Oryza sativa* Linn. (Poaceae) absorbed silver nanoparticles (AgNPs), with diameter varying from 20 to 150 nm (Thuesombat et al. 2014). Treatment of *Cucurbita pepo* Linn. (Cucurbitaceae) with superparamagnetic iron oxide NPs, about 12.5 nm in size, also showed that these particles pass the cell wall (Tombuloglu et al. 2019a, b). Moreover, it was shown that zinc oxide NP (ZnO-NP) of 8 nm in diameter may enter cytoplasm of root cells of *Brassica sp.* Linn. (Brassicaceae) species (Molnár et al. 2020). None of the works mentioned above explores the main question related to the passage of molecules through the cell wall: what mechanism allows NPs to cross the cell wall barrier?

Although the mechanisms of NMs passage through the cell wall are not yet completely understood, it is almost unanimous in the literature that the change in the chemical composition of the cell wall is involved in this process (Kurczynska et al. 2021). Such change, related to response to biotic and abiotic factors, are increasingly investigated as an adaptation mechanism to altering environmental conditions (Milewska-Hendel et al. 2017). Chemical composition of the cell wall influences its structure, including porosity. This parameter in turn determines the pore size of walls and controls the apoplastic exchange of macromolecules (Kurczynska et al. 2021). Recent studies also elucidated the cell wall as a dynamic structure during secretion passage on secretory structures (Miguel et al. 2006, 2017) and silver NPs passage (Paiva Pinheiro et al. 2021).

Until recently, the most accepted mechanism for the passage of NMs through cell walls involved the rupture due to NPs accumulation on the cell wall surface. Most parts of these studies were conducted in the bacteria, fungi, and microalgae cell wall (Concha-Guerrero et al. 2014; Athie-García et al. 2018; Wang et al. 2019).

A detailed mechanism was proposed for the passage of AgNPs through the cell wall of *Lactuca sativa* var. crispa Linn. (Asteraceae) and occurs in three steps: AgNPs contacts the outer cell wall, presenting two states of oxidation, silver ion (Ag^+ and Ag^0); the interaction of oxygen with the Ag^+ leads the rupture of hydrogen bonds on cellulose microfibrils; hydrogen bonds break spaces large enough to AgNPs freely passage through the outer cell wall (Paiva Pinheiro et al. 2021). This work was the first to convincingly elucidate the mechanism of passage of AgNPs through the cell wall using *L. sativa* as a model. However, the surface chemistry of ENMs is also very important as it may influence NPs reactivity, penetration and movement within the plant and therefore plant responses to the same type of NPs may be completely different (Zhu et al. 2012; Wang et al. 2014; Li et al. 2016; Milewska-Hendel et al. 2019).

As discussed, it is likely that other plants exhibit peculiarities in the passage of metallic NPs through the cell wall, however, this mechanism seems quite attractive since it explains the process based on the behavior of cellulose microfibrils, common to all cell walls of higher plants. After crossing the entire cell wall barrier, before entering the cytoplasm, the nanostructured materials must pass through the cell membrane.

ENMs cellular uptake has been mainly studied in animal cells (see Zhao et al. 2011; Foroozandeh and Aziz 2018). Different endocytic pathways seem to be responsible for the internalization of these materials, however, these results cannot be used as models to plant cells, due to the presence of the cell wall, which became an additional barrier outside the plasma membrane (Palocci et al. 2017).

Plant uptake of ENMs is hardly predictable. It depends on multiple factors including the NP itself (size, chemical composition, net charge and surface functionalization); the application routes; and the interactions with environmental components (soil texture, water availability, microbiota) (Sanzari et al. 2019). In addition, anatomical and ultrastructural variations of each species must also be considered. Different plant cell membranes, e.g., plasmalemma and plasma membrane, are known for selective absorptivity and permit small nonpolar ions diffusion through the membrane (Ghorbanpour and Wani 2019). Larger and polar molecules such as water, ions, and other foreign particles (including ENMs) cannot easily diffuse across the phospholipid bilayer. Although this process is not simple, many ENMs are able to break through this barrier and enter the cell.

Several mechanisms have been proposed for ENMs uptake in plant cells, e.g., by endocytosis, binding to carrier proteins, through aquaporins, ion channels, creating new pores, or by binding to organic chemicals in the environmental media (Rico et al. 2011). The most investigated mechanism for cellular uptake of ENMs in plant cells is endocytosis. The internalization of ENMs depends on the interaction established with the plasma membrane and the type of endocytosis is associated with physicochemical characteristics of NMs (size, chemical composition, charge, surface functionalization, surface reactivity and surface adsorption) (Jiang et al. 2011). Endocytosis can be classified as phagocytosis, which involves the internalization of particles larger than 1 µm and pinocytosis involves the internalization of particles smaller than 500 nm (Costa Verdera et al. 2017). Pinocytosis can further be classified as clathrin-mediated endocytosis (CME), caveolin-mediated endocytosis (CAV), macropinocytosis, clathrin-independent endocytosis (CIE) and caveolin-independent endocytosis (CIEV) (Yameen et al. 2014). CME was identified as the dominant endocytic process in plant cells and appears to operate analogously to animal cells (Surpin and Raikhel 2004). CME involves the formation of vesicles from the polymerization of the protein clathrin that acts as a transporter of several compounds to the cell across the plasma membrane and released into the cytoplasm (Costa Verdera et al. 2017). Studies show that the size limit of particles entering cells via CME has been reported to be around 200 nm (Rejman et al. 2004; McMahon and Boucrot 2011).

Caveolin-mediated endocytosis is another mechanism that transports NPs across the plasma membrane to the cell. These caveolae are membrane invaginations, enriched with cholesterol and sphingolipids (Lajoie and Nabi 2007). Several reports suggest that caveolae sizes limit the uptake of NPs larger than individual caveolae sizes (approximately 50–100 nm) (Wang et al. 2012; Akinc and Battaglia 2013).

Macropinocytosis involves the transport of large amounts of extracellular material through vesicles known as macropinosomes. Macropinosomes provide an effective pathway for endocytosis of macromolecules, as their structure is substantially larger with a diameter of 0.5–10 µm (Falcone et al. 2006). Clathrin and caveolin-independent endocytosis do not use the classic routes of internalization, but they are responsible for the transport of large amounts of extracellular material. A CIE and CAIE do not require the presence of coat proteins for the formation of vesicles and internalization of extracellular material (Sandvig et al. 2008). Most of studies on plant endocytosis were performed in suspension cells, which do not recapitulate tissue structure and have been reported to possess half-plasmodesmata that expose the cell membrane to the extracellular environment (Bayer et al. 2004). For these reasons, information about this topic must be taken with care. When crossing the cell membrane, NPs are transported to the cell target by several mechanisms.

Once in the cytoplasm, cell to cell movements of ENMs are facilitated by plasmodesmata, membrane-lined cytoplasmic bridges with a flexible diameter that ensure membrane and cytoplasmic continuity among cells throughout plant tissues. Transport of ENMs with variable sizes through plasmodesmata has been described in *Oryza sativa* (Lin et al. 2009), and poplar plant species (Zhao et al. 2017). Through the symplastic and apoplastic pathways, small particles can reach the xylem and phloem vessels and translocate in the whole plant to different tissues and organs. Remarkably, organs like flowers, fruits and seeds normally have a strong capability to import fluids from the phloem (sink activity) and tend to accumulate NMs.

In the cell, the address of these NMs is directly related to their characteristics such as size, charge, and pH. According to the characteristic of the ENMs they are endocytosed by different pathways (Jiang et al. 2011). After entering the cell, the ENMs acquire lipoprotein coverage (Sahay et al. 2010) and follow the classic route that begins with the fusion of ENMs with early endosome, then it becomes a late endosome and finally the transfer of the material to the lysosomes (Lab and Jesus 2014). Until reaching lysosomes, NMs go through several maturation processes and decrease their pH and this traffic is regulated by RAB proteins (Family G-proteins) (Yameen et al. 2014).

ENMs can follow an alternative route, a fact related to their characteristics and hence the importance of characterizing these ENMs. The most usual alternative to scape endosomal degradation is related to the alteration of the NPs surface charge, the surface functionalization of NPs with an amine (Ogris et al. 2001). The fate of NPs in the cell can be in a specific way with surface functionalization or in a non-specific way for different organelles such as the nucleus, Golgi apparatus, mitochondria and endoplasmic reticulum (Yameen et al. 2014). An accurate analysis regarding ENMs plant interactions concern not only the degree of toxicity of ENMs on living organisms, but also detailed studies of their uptake and movement within the plant body on different levels of organization: organs, tissues and cells (Kurczynska et al. 2021). These ENMs are transported via apoplast to the endodermis and via symplast to the vascular system (Pariona et al. 2017). Xylem is the most significant carrier in the dissemination and transfer of ENMs (Aslani et al. 2014) and studies show the rapid absorption of NPs through conductive vessels.

A study with different crop plants showed that after 24 h of exposure of the root to bioferrofluid (magnetic carbon-coated NPs), the NPs were able to leak into vascular tissues and reach the aerial parts of the plants (Cifuentes et al. 2010). In another study, it was observed that iron NPs (IO-NPs) were absorbed by the roots of maize seedlings and through the vascular system reached the shoot and accumulated in the leaves (Pariona et al. 2017). A similar result was observed by Tombuloglu et al. (2019a) in barley plants.

Furthermore, the translocation of NPs can occur in the reverse way, from phloem to xylem. González-Melendi et al. (2008) showed that the translocation of NPs applied in the aerial part of plants went to the roots and there is evidence that cell–cell transport occurs (Corredor et al. 2009) which may involve the traffic path through the plasmodesmata (Cifuentes et al. 2010). A similar result was observed by Wang et al. (2012) in which copper NPs (CuONPs) were found in the xylem of maize seedlings, indicating xylem-mediated transfer of NPs from root to shoot. In addition, reverse movement through the phloem.

Although osmotic pressure favors the uptake and translocation of NPs, the NPs can accumulate in the root and not be translocated throughout the plant (Iannone et al. 2016). As mentioned earlier, absorption, endocytosis and translocation will depend on the characteristics of the ENMs and the type of plant species used in the study. Figure 5.1 shown a schematic representation of uptake and traffic of ENMs on plant cells.

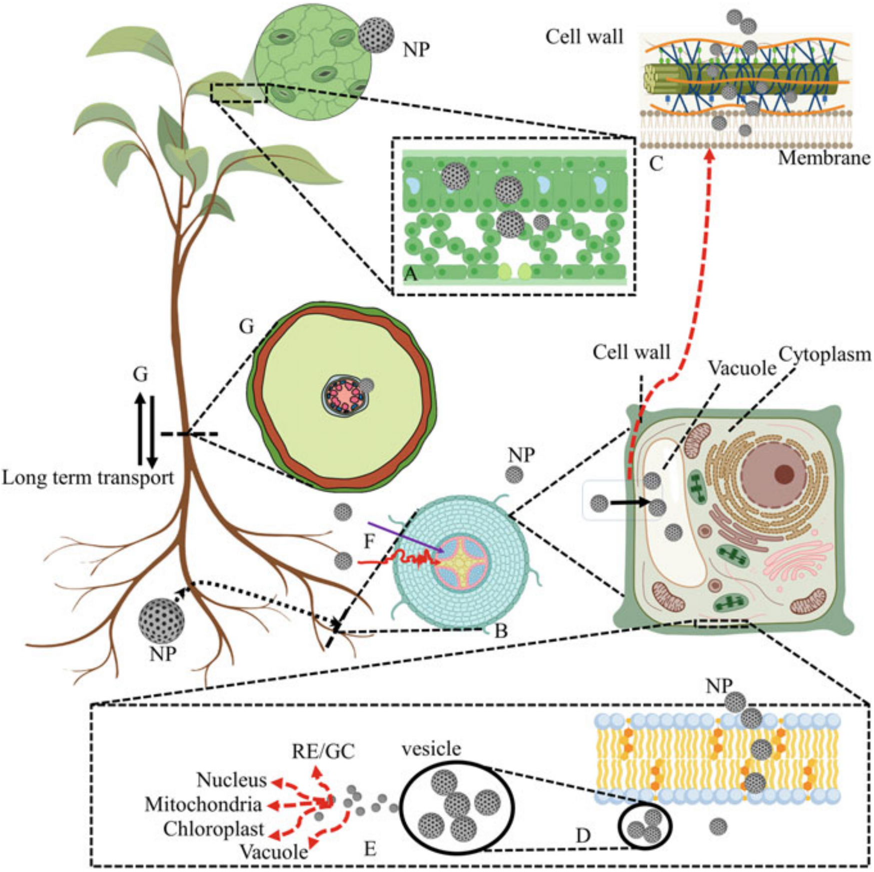


Fig. 5.1 Schematic representation of ENMs uptake and traffic on plant cells. ENMs contact plants in different ways. The nanostructured material can come into contact through the leaf (**a**) or root (**b**). Regardless of the contact route, the first barrier that must be overcome for the internalization of NMs in the cell wall (**c**). Then, to enter the symplast pathway, it is necessary to pass through the cell membrane (**d**). Subsequently, ENMs can be delivered to different organelles, either by vesicles or free in the cytoplasm (**e**). Sometimes the particles continue their apoplast movement until they reach the endoderm and pericycle, are forced into the symplast pathway reaching the xylem and phloem cells (**f**). If this happens, long term transport through vascular tissue (**g**) may be possible. The sequence of events, from the interaction between NMs and cell walls to long term transport, is a particular result of the interaction of each particle and plant, in addition to the concentration and route of exposure. Therefore, these results are unpredictable most of the time. A and B– ENMs contact to cell wall; C—Accumulation and passage through the cell wall; D—Passage through the cell membrane; E—Transport, traffic and targeting; F—Apoplast transport and G—Long distance transport. (Figure constructed by the authors)

5.3 How Do Nanomaterials Interacts with Photosynthetic Pigments?

For photoautotrophic organisms, physiological and/or ecological functions, such as energy absorption, antioxidant activity, protective or reproductive processes, and some others, are related to a large range of molecules categorized as pigments. The most important contribution of natural pigments is related to Earth's biosphere maintenance due to their role in the water-splitting activity responsible for oxygen photodissociation.

Photosynthesis (from Greek photo → light and synthesis → putting together) is one of the most important processes on Earth and spawns a research field that is intrinsically interdisciplinary. From ancient environments (ca. 2.8–2.4 billion years ago), when the first oxygenic cyanobacteria began to utilize the photosynthetic process until the evolution of land-based plants, the driving selective forces acted to oxidize the water molecule and fix carbon in high amounts (Melkozernov 2014; Stirbet et al 2014). As a consequence of their organic evolution, some molecules with phenotype chromophore properties were promoted by natural selection, i.e., compounds with high absorption at the visible portion of the electromagnetic spectrum.

In oxygenic photosynthesis of higher plants, electrons are extracted from water by solar energy (photons) absorption followed by their incorporation into CO_2 to synthesize organic compounds, such as sugars, as storable chemical energy ($NADPH^+$ and ATP).

Chloroplasts (Fig. 5.2) are bioenergetic plant cell organelles of diameter 5–10 μm. These structures are responsible for photosynthesis, with each plant cell, mainly leaves, containing about 10–100 chloroplasts (Cooper 2013). Chloroplasts comprise two membranes: an outer membrane that delimits the organelle and an inner membrane related to inner functions. Between the outer and inner membrane, a space of 10–20 nm defines its intermembrane space. In the inner organelle portion is the chloroplast stroma, a soluble matrix of dense fluid and an internal membrane system of layered thylakoids, called "grana" (Cooper 2013).

Pigments are the part of the macromolecular structures that absorbs light in the visible region of the spectrum (Walsby 1974). A large range of pigments can be found in different photosynthetic organisms such as chlorophylls (Chl) *a*–*f*, carotenoids (Car), bacteriochlorophylls (B-Chl) *a*–*g*, and bilins (phycoerythrin, phycocyanin, and allophycocyanin).

In summary, Chl-*a* molecules are primarily responsible for photochemical reactions. ENMs can interact differently with these and other plant pigments according to their physical–chemical properties and pigment type, resulting in two basic responses: changes in the pigment content (increase/decrease) by induction of the pigment synthesis or its inhibition and/or degradation and changes in the pigment activities, especially light absorption and energy dissipation processes such as the behavior of fluorescence emission, electron transport, and others related to non-photochemical quenching. In general, when plants are exposed to NPs, the most

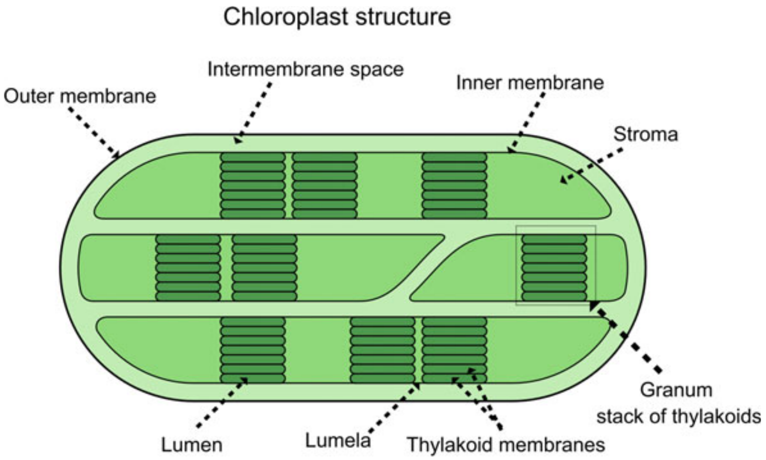


Fig. 5.2 Schematic representation of chloroplast structure. Chloroplasts are semiautonomous organelles in plants, algae, and cyanobacteria cells. Light energy is transduced into chemical energy at the thylakoid membrane, and the fixation of CO_2 takes place in the stroma side. (Figure constructed by the authors)

significant effect described in the literature is the reduction of chlorophyll content followed by an increment in the amount of accessory pigments (Table 5.1).

5.3.1 *Chlorophyll and Derivatives*

Chlorophyll (Chl) pigment is a green-colored, lipid-soluble porphyrin derivative with magnesium (Mg) as the central atom; it is found in all photoautotrophic organisms (Mulder-Krieger and Verpoorte 1994; Cooper 2013; Kuczynska et al. 2015). The chlorophylls are located into chloroplast organelles and play a crucial role in photosynthetic light reactions. A wide range of chlorophyll forms (*a*–*f*) can be found in different photosynthetic organisms, depending on their functional groups (methyl, ethyl, formyl, vinyl). Chlorophyll-*a* and chlorophyll-*b* molecules (Fig. 5.3) are primarily responsible for photochemical reactions in higher plants.

In the last decades, it has become increasingly interesting to understand the interaction of ENMs and photosynthetic pigments for environmental impact monitoring purposes or for the development of novel materials for artificial photosynthesis. For instance, changes in pigment activities have been described as variations on the excited-state of chlorophyll-a interacting with gold NPs (AuNPs) (Barazzouk et al. 2012; Falco et al. 2011; Torres et al. 2018; Mezacasa et al. 2020).

Under excitation energy (blue and red light around 680 nm and 760 nm, respectively), the chlorophyll molecule may oscillate between different energy states, with electronic transitions from highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO) (Müh and Renger 2014), dissipating part

Table 5.1 Effects in the pigment content of plants exposed to nano-metal particles

NP	Size (nm)	Organism	Pigment content decrease	Pigment content increase	Refs.
Silver (Ag)	20	*Arabidopsis thaliana* Linn. Heynh (Brassicaceae)	Chl (total)	Anthocyanin	Nair and Chung (2014)
	2–11	*Brassica rapa* Linn. (Brassicaceae)	Chl (total)	Anthocyanin	Thiruvengadam et al. (2015)
	23 ± 4	*Chrysanthemum grandiflorum* Ramat. (Asteraceae)	n.a	Carotenoids	Tymoszuk and Kulus (2020)
	10–30	*Lactuca sativa* Linn. var. foliosa (Asteraceae)	n.a	Carotenoids, and increase Chl *a* content	Jurkow et al. (2020)
	100	*Stevia rebaudiana* Bertoni (Asteraceae)	n.a	Anthocyanin and Chl (total)	Ramezani et al. (2019)
	58–80	*Viola tricolor* Linn. (Violaceae)	n.a	Rutin, Luteolin, Apigenine, and Quercetin	Hassanvand et al. (2021)
	14	*Withania coagulans* Stocks (Solanaceae)	Anthocyanin—dose dependent	Chlo *b*, and Carotenoids—dose dependente	Tripathi et al. (2020)
	10	*Wolffia globosa* den Hartog & van der Plas (Araceae)	Chlo *a* and Carotenoids	n.a	Zou et al. (2016)
Arsenic trioxide (As_2O_3)	20	*Hordeum vulgare* Linn. (Poaceae)	Chl (total)	Anthocyanin and Carotenoids	Selim et al. (2021)
	20	*Zea mays* Linn. (Poaceae)	Chl (total)	Flavonoids	Selim et al. (2021)
Gold (Au)	5–30	*Chlorella zofingiensis* Linn. (Chlorophyceae)	n.a	Carotenoids	Li et al. (2020)

(continued)

Table 5.1 (continued)

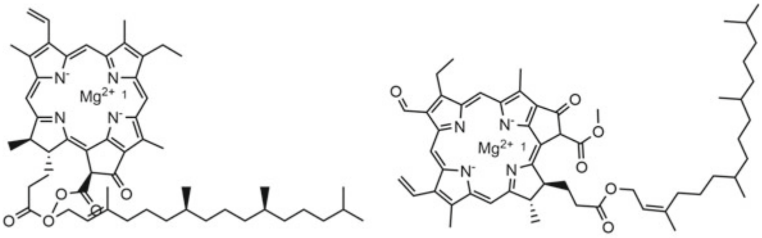
NP	Size (nm)	Organism	Pigment content decrease	Pigment content increase	Refs.
	10–30	*Lactuca sativa* Linn. var. foliosa (Asteraceae)	n.a	Carotenoids	Jurkow et al. (2020)
Cerium oxide (CeO_2)	8	*Phaseolus vulgaris* var. Red Hawk Linn. (Fabaceae)	Chl *a* and Carotenoids	n.a	Majumdar et al. (2016)
	130	*Salvinia auriculata* Aubl. (Salviniaceae)	Chl (total)	Carotenoids	Pontes et al. (2019)
Copper oxide (CuO)	25–55	*Brassica rapa* var. rapa Linn. (Brassicaceae)	Chl (total) and Carotenoids	Anthocyanin	Chung et al. (2019)
	120	*Lemna valdiviana* Phil. (Lemnaceae)	Chl *a*	n.a	Pontes et al. (2020)
	50	*Oryza sativa* Linn. (Poaceae)	Chl (total) and Carotenoids	n.a	Yang et al. (2020)
	25–55	*Solanum melongena* Linn. (Solanaceae)	Chl (total)	Anthocyanin and Flavonoids—dose- dependent	Baskar et al. (2018)
	30–40	*Zea mays* Linn. (Poaceae)	Carotenoids	Anthocyanin and Chl (total)	Nguyen et al. (2021)
Iron oxide (Fe_2O_3)	100	*Trachyspermum ammi* Linn. (Apiaceae)	Chl *a*	Anthocyanin, Carotenoids and Chl *b*	Abdoli et al. (2020)
Nikel (Ni)	10 - 20	*Solanum melongena* Linn. (Solanaceae)	Chl (total)	Anthocyanin and Flavonoids—dose- dependent	Baskar et al. (2018)
Silicon oxide (SiO_2)	10–20	*Scenedesmus obliquus* (Turpin) Kuetzing. (Scenedesmaceae)	Chl (total)	n.a	Wey et al. (2010)

(continued)

Table 5.1 (continued)

NP	Size (nm)	Organism	Pigment content decrease	Pigment content increase	Refs.
Platinum (Pt)	10–30	*Lactuca sativa* L. var. foliosa (Asteraceae)	n.a	Carotenoids	Jurkow et al. (2020)
Titanium oxide (TiO_2)	10–30	*Lycopersicum esculentum* Mill (Solanaceae)	Chl (total)	Anthocyanin Carotenoids	Ko and Hwang (2019)
Zinc oxide (ZnO)	30	*Carex vulpina* Linn. (Cyperaceae)	Chl (total), Neoxanthin and Lutein	β-carotene, Violaxanthin and Zeaxanthin	Haisel et al. (2019)
	18	*Solanum melongena* Linn. (Solanaceae)	Chl (total)	Anthocyanin and Flavonoids—dose—dependent	Baskar et al. (2018)

n.a. = not applicable



Chlorophyll *a* Chlorophyll *b*

Fig. 5.3 Chlorophyll *a* and *b* structures with a magnesium as the central atom. (Figure constructed by the authors)

of the primary absorbed energy to the electron transport chain (photochemical process) and another part via the non-photochemical or quenching process (heat and fluorescence) (Roháček 2002; Ashraf and Harris 2013).

Nanoparticles can interfere with the regular behavior of chlorophyll, reducing its fluorescence dissipation. This occurs either via a static process, i.e., interaction with the fluorophore molecule (chlorophyll) to form a non-fluorescent complex or via dynamic processes, i.e., when the NPs acting as a quencher, reducing the fluorescence intensity by fluorophore deactivation; this suppression is directly related to particle size and is dose-dependent (Acquavella et al. 1995; Sharma et al. 2019; Damera et al. 2020). On the other hand, up-converting and down-converting NPs were able to transform infrared light into visible light or ultraviolet light into visible light, respectively (Yanykin et al. 2022). These NPs can be exploited to improve photosynthesis.

In general, AuNPs suppress the electron transfer process from excited chlorophyll molecules. Additionally, the photodegradation behavior of Chl-a can be slowed during its interaction with AuNPs (Barazzouk et al. 2012) and this information provides important insight for the development of efficient hybrid artificial photosynthesis. Interaction between chlorophyll and silver NPs (AgNPs) was investigated by Falco et al. (2015) and Queiroz et al. (2016), who showed that AgNPs can disrupt photosynthetic electron transport via excited electron transfer from the chlorophyll molecules to the AgNPs surface (Fig. 5.4). Additionally, this behavior was dependent on NPs size and concentration.

Recently, the temperature-dependent interaction behavior of chlorophyll with zinc oxide, copper oxide, titanium dioxide, and iron oxide NPs was studied (Sharma et al. 2019, 2020). These studies also observed that electrons can be relocated from the excited chlorophyll to the conduction band of the surface of the NP. However, the authors suggest that with the temperature increase, hydrogen bonding and van der Waals forces are the prominent driving forces during this interaction, and this process is spontaneous and exothermic.

Under in vivo cellular media, chlorophyll molecules are susceptible to losses of their Mg atom (magnesium dechalatase) due to low pH or heat conditions. This reaction is called "pheophytinization" and results in pheophytin formation when two

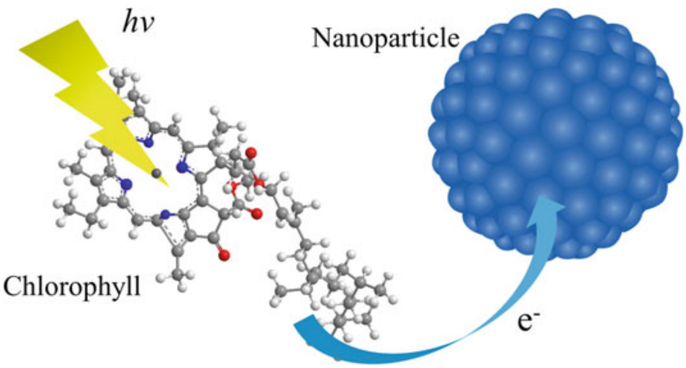


Fig. 5.4 Schematic representation of excited electron transfer from chlorophyll molecule to nanoparticle surface. *hv*: is the energy of a photon, and e^-: is the excited electron. (Figure constructed by the authors)

hydrogen ions replace the Mg ion found in the center of the porphyrin ring (Sato et al. 2018). Pheophytin plays a key role in Photosystem II (PSII) electron transport. Structurally, D1 and D2 proteins contain special chlorophyll P680, chlorophyll-a, and pheophytin in the reaction center of PSII (Santiago et al. 2020; Pontes et al. 2020). Chlorophyllase enzyme may be binding with the phytyl group of pheophytin, resulting in pheophorbide formation. Equation 1 summarizes the formation of chlorophyll derivatives [Eq. 1]. Size dependence was studied using in vitro and theoretical experiments to understand the interaction of AuNPs and pheophytin (Mezacasa et al. 2020). The interaction mechanisms reported for pheophytin and pheophorbide molecules are also similar to chlorophyll via photoinduced electron transfer from pheophytin or pheophorbide molecules to AuNPs surface (Mezacasa et al. 2020; Kotkowiak and Dudkowiak 2015).

$$\text{Chlorophyll} \rightarrow \frac{-Mg^{+2}}{Acid/heat} \rightarrow Pheophytin \rightarrow \frac{-phytol}{Chlorophyllase} \rightarrow Pheophorbide \tag{5.1}$$

The impact of the nanomaterial's interaction on plant photosynthetic pigments, particularly with chlorophyll and its derivates, may alter the cellular homeostasis, metabolism, physiology, and the plant life cycle. Consequently, this either positively or negatively modifies plant productivity.

5.3.2 Carotenoids

Carotenoids (Car) are a class of natural pigments with important roles in biological systems. In photosynthesis, Car act as accessory light-harvesting pigments and can efficiently transfer absorbed light energy to chlorophyll molecules (Zulfiqar et al. 2021). Additionally, another important role of the Car at the thylakoid membranes during photochemistry is photoprotection by dissipating excess energy to the environment (Collini 2019). In general, carotenoids can be divided into two major groups based on their chemical structure: cyclic hydrocarbons (carotenes) and hydrocarbons containing oxygen (xanthophylls) (Jan and Abbas 2018).

The interaction of NPs with carotenoids may be affected by several factors related to the nature of the nanoparticle, the type of carotenoid molecule, and the interaction of the NMs with the cellular environment (Santiago et al. 2020; Miguel et al. 2021). For instance, Heisel et al. (2019) report an increase in negative effects of cadmium contamination in *Carex vulpina* Linn. (Cyperaceae) plants exposed to ZnO NPs. These authors observed a strong decrease in the neoxanthin and lutein content, suggesting damages to light-harvesting complexes, also, increased β-carotene content suggests stress behavior.

Once the NPs penetrate the plant chloroplast and interact with carotenoids, complexation of the nanomaterial and pigment may occur. For instance, the strong complexation of carotenoids containing terminal carboxyl groups (-COOH) with the TiO_2 surface leads to electron transfer from the adsorbed carotenoid molecule to the surface trapping site (Wang et al. 2005). Carboxyl groups enhance the binding of carotenoids onto the surface of TiO_2 NPs. This strong attachment can effectively facilitate electron transport from excited carotenoids by injecting electrons from their excited states into the conduction band of TiO_2 NPs (Wang et al. 2005). Additionally, photosensitization of the TiO_2 NPs with β-Carotene, 8′-apo-β-carotene-8′-al, and canthaxanthin leads to the formation of superoxide anion (O_2^-) and singlet oxygen ($^1O_2$) on red light irradiation (Konovalova et al. 2004) as schematized in Fig. 5.5.

On the other hand, according to nanoparticle size, AuNPs may act as light-harvesting NPs and demonstrate the enhanced biosynthesis of carotenoids — 42.7% higher than without light-harvesting gold NPs (Li et al. 2020). In vivo studies have shown that the characteristics and nature of the NMs, in addition to the environmental factors, will greatly influence the synthesis and accumulation of carotenoids in plant tissues (Zou et al. 2016; Manjudar et al. 2016; Pontes et al, 2020). According to Pan et al. (2004), carotenoids with pheophytin molecules in contact with metal oxide NPs may form a self-assembled system, leading to an efficient reductive quenching of the pheophytin moiety. This result suggests that a similar mechanism can operate also in natural photosynthetic systems. Additionally, the impact of NMs' interaction on carotenoids pigments may alter the plant physiological responses (Boonlao et al. 2022). At the reaction center, carotenoids can play the role of electron donor when a suitable electron acceptor is available. They can also effectively quench chlorophyll

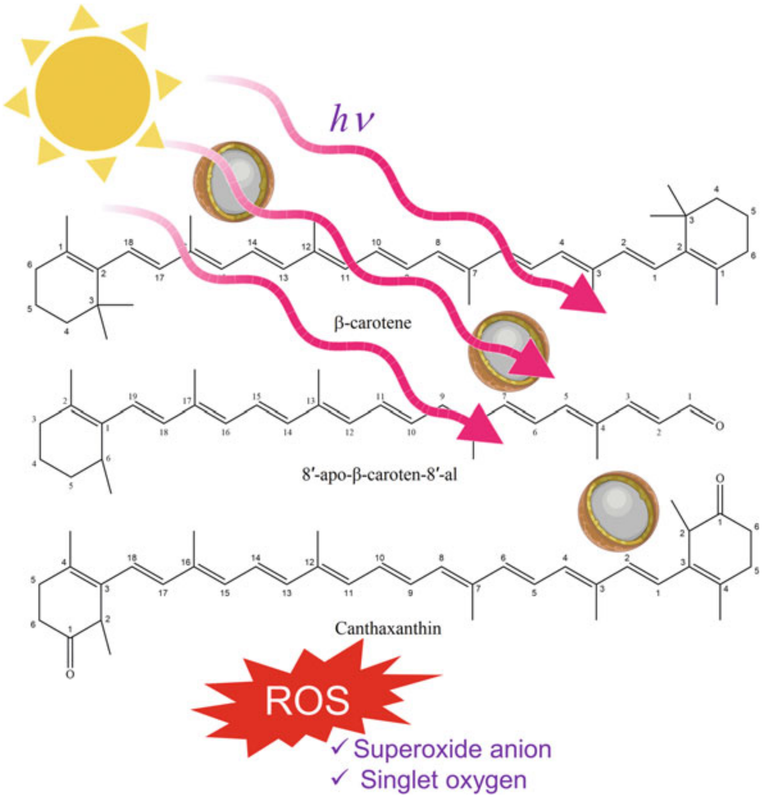


Fig. 5.5 Generation of superoxide anion and singlet oxygen in irradiated TiO_2 nanoparticles during its interaction with carotenoids. *hν*: is the energy of a photon. (Figure constructed by the authors)

triplet states and the formed triplet states of carotenoids can harmlessly dissipate the excess energy to the environment.

Despite many studies conducted experimentally with several plant model organisms on nanomaterial exposure, the disparity in results between physiological and molecular in vivo studies is remarkable (Santiago et al. 2020; Costa-Ruiz et al. 2021). Even if nano-enabled agriculture or artificial solar cells technologies show us success stories in the areas cited above, these new areas still have major challenges and limitations that must be overcome to achieve the desired outcome, particularly in terms of the interaction between NMs and non-chlorophyll pigments.

5.4 Anthocyanins

Anthocyanins are a class of natural red pigments present in leaves, petals, stamens, tuberous roots, and particularly in vacuoles of red-colored epidermis cells (Fang, 2014). Anthocyanins act as a cell protection agent and are considered antioxidant molecules (Gould et al. 2002). Biotic and abiotic stress, such as herbivory, high temperatures, and ultraviolet radiation, can trigger changes in anthocyanin content (Hatier and Gould, 2009), and can induce the generation of reactive oxygen species (ROS) in the plant cells. Consequently, increases the expression of MYB, basic helix-loop-helix (bHLH), and WD40 genes involved in the anthocyanin biosynthesis pathway (Tahara, 2007).

The interaction between NMs and anthocyanins may occur on isolated compounds (in vitro assays) and/or at a cellular/biochemical level as described by Ravanfar et al. (2016) who has researched solid lipid NPs as carriers for anthocyanins. A recent study by Amin et al. (2017) demonstrated that anthocyanins loaded onto polyethylene glycol (PEG) nanocapsules can decrease oxidative stress on the p38/c-Jun N-terminal kinases (JNK) pathway of SH-SY5Y cells. In general, the extraction methods for anthocyanin usually require complex enzymatic procedures of extraction and higher costs, and are time-consuming. On the other hand, nanobiocatalysis using nanomaterial functionalized with α-amylase demonstrated a higher efficiency for anthocyanin extraction (Yi et al. 2021). For instance, after NP internalization in biological systems, the nanomaterial directly interacts with reactive molecules (Miguel et al. 2021). In *Arabidopsis* leaves, TiO_2 NPs can induce cell membrane damage and anthocyanin capping the surface of TiO_2NPs, forming a pigment-layer, which is responsible for the blue color of internalized NPs (Kupera et al. 2020).

Another factor that has an impact on nanoparticle–anthocyanin interaction is the synthesis route of the nanomaterial and the anthocyanin synthesis pathway (Fig. 5.6). According to Ramezani et al. (2019), nanosilver synthesized via the green route using *Stevia rebaudiana* Bertoni (Asteraceae) extracts increased the synthesis of anthocyanin when compared with chemically synthesized nanosilver. On the other hand, Tripathi et al. (2020) revealed a dose-dependent decrease in anthocyanin content in *Withania coagulans* Stocks. (Solanaceae) in response to biogenic nanosilver. Hassanvand et al. (2021) reported increased gene expression of flavonoid biosynthesis pathway via phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) genes. In this study, Hassanvand and co-workers, using *Viola tricolor* Linn. (Violaceae) plants exposed to AgNPs, also observed an increase in the content of anthocyanin. This finding corroborates with the increase observed in the activity of phenylalanine ammonia-lyase and flavonol synthase enzymes in *Brassica rapa* Linn. (Brassicaceae) plants exposed to CuO NPs (Chung et al. 2019). Additionally, *Chrysanthemum grandiflorum* Ramat. (Asteraceae) inflorescence colors are affected by silver NPs due to alterations to gene expression; hence, changes in anthocyanin amount are responsible for the phenotypic response (Tymoszuk and Kulus 2020).

Nano-enabled agrochemicals have shown prominence in the modern agri-food sector; hence it has become important to understand the metabolic interaction at the

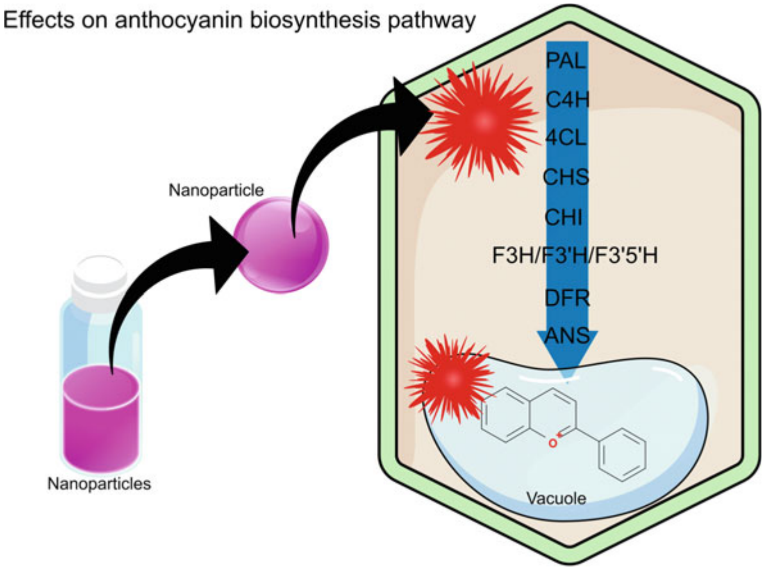


Fig. 5.6 Impact of engineered nanomaterials on the anthocyanin biosynthesis pathway. PAL: phenylalanine ammonia lyase; C4H: cinnamate 4-hydroxylase; CHS: chalcone synthase; CHI: chalcone isomerase; F3H/F3'H/F3′5'H: flavanone-hydroxylases; DFR: dihy- droflavonol 4-reductase; and ANS: anthocyanidin synthase. (Figure constructed by the authors)

interface between NMs and plant pigments. With regard to secondary metabolism in plants, special attention has been given to flavonoids such as anthocyanins, especially changes in these pigment levels, which provide a reliable tool to evaluate the plant response to engineered NMs. Soybean plants under hydric stress and nanosized copper particles show an increase in anthocyanin production due to ROS response (Nguyen et al. 2021). These results suggest that CuONPs may alleviate hydric stress in soybean plants. In *Solanum melongena* Linn. (Solanaceae) plants exposed to metal oxide NPs, the anthocyanin amount increases with NPs uptake by plants (Baskar et al. 2018). Nanosilver exposure increased the anthocyanin content in *Brassica rapa* Linn. (Brassicaceae) (Thiruvengadam et al. 2015) and gold NPs also increased the anthocyanin content in *Arabidopsis* plants (Nair and Chung 2014). This behavior occurs probably due to the ROS generation induced by NPs exposure (Santiago et al. 2020).

Photosynthetic groups of C3 and C4 plants respond differently to environmental fluctuations. For instance, under the high availability of CO_2, plants tend to increase their anthocyanin content as an antioxidant molecule. In co-exposure of CO_2 with AS_2O_3 NPs on the C3 plant *Hordeum vulgare* Linn. (Poaceae), Selim and co-workers (2021) demonstrated an accumulation of anthocyanin in C3 plants in contrast with C4 plants *Zea mays* Linn. (Poaceae) that show low content of its pigment. In Pb-contaminated soils, silicon NPs regulate the anthocyanin amount in *Coriandrum sativum* Linn. (Apiaceae) plants in soils with elevated Pb levels (Fatemi et al.

2021). Also, exposure of *Trachyspermum ammi* Linn. (Apiaceae) plants to iron NPs improves the anthocyanin content under salinity stress (Abdoli et al. 2020). Another synergic behavior against salinity stress was observed by Gohari et al. (2021). Using carbon quantum dots in *Vitis vinifera* Linn. (Vitaceae) plants, these authors observed a decrease in anthocyanin content, probably due to changes in its biosynthesis pathway. Finally, the application of anthocyanin for dye-sensitized solar cells (DSSCs) has shown promising potential. For instance, DSSCs made from blackberry-based anthocyanin-capped TiO_2 NPs to give the largest photovoltaic output and this photovoltaic output compares well with silicon-based solar cells due to its high efficiency (Cramer et al. 2011).

5.5 Betalains

The betalains is another important and interesting group of water-soluble nitrogenous plant pigments and have been studied by photoelectrochemical assays (Zhang et al. 2008; Gandia-Herrero and Garcia-Carmona 2013). Particularly important is their interaction with engineered semiconductor NMs to design novel dye-sensitized solar cells, due to their eco-friendly and low cost of production. Betalains are divided into two classes: yellow betaxanthins and red–violet betacyanins (Zhang et al. 2008). Additionally, betalain pigments show higher pH-dependent redox properties.

According to Wendel et al. (2017), betalains show limited sunlight energy conversion efficiency for applications in environmentally friendly dye-sensitized solar cells (DSSCs). This insufficient electron injection quantum yield is the major reason for these phenomena. On the other hand, some studies reveal that the interaction of ENMs improves their sunlight energy conversion efficiency. For instance, a DSSC prepared with betalains adsorbed onto Ag and TiO_2 nanostructure surfaces yielded a better plasmonic-enhanced DSSC, giving a short-circuit current density (Jsc), fill factor (FF), and power conversion efficiency (PCE). In general, these modified DSSCs increase efficiency by 50% over the reference DSSC (Isah et al. 2016).

The role and relevance of betalains' light emission in flowers and fruits in attracting pollinators and/or seed dispersers is a matter of current ecological and biophysical debate (Gracía-Plazaola et al. 2015; Mori et al. 2018; Guerrero-Rubio et al. 2019). For instance, nanosilver has a phytostimulatory effect on flowering (Salachna et al. 2019). However, knowledge of the interaction of NMs with flower pigments and their pollinators is needed to better understand their ecological impact (Hooven et al. 2019).

In general, under natural environments, for seeds to grow and develop into new plants, they must be dispersed. The possible influence of ENMs on the fluorescence of betalains in seed dispersal may have biological relevance and needs to be investigated in further studies. For instance, quinoa grains contain a significant amount of betaxanthins and their levels can affect the vigor of the seeds (Escribano et al. 2017; Guerrero-Rubio et al. 2019). Thus, the discussion about the effects of NMs on fluorescence and colors of seeds opens up to include a possible biological effect of nanostructures on these signals and, consequently, on seed dispersal behavior.

Consequently, the interaction mechanisms of nano-based materials with betalains can and will play an important role in the future of artificial photosynthesis, thus requiring a better understanding of their ecological safety (Fig. 5.7).

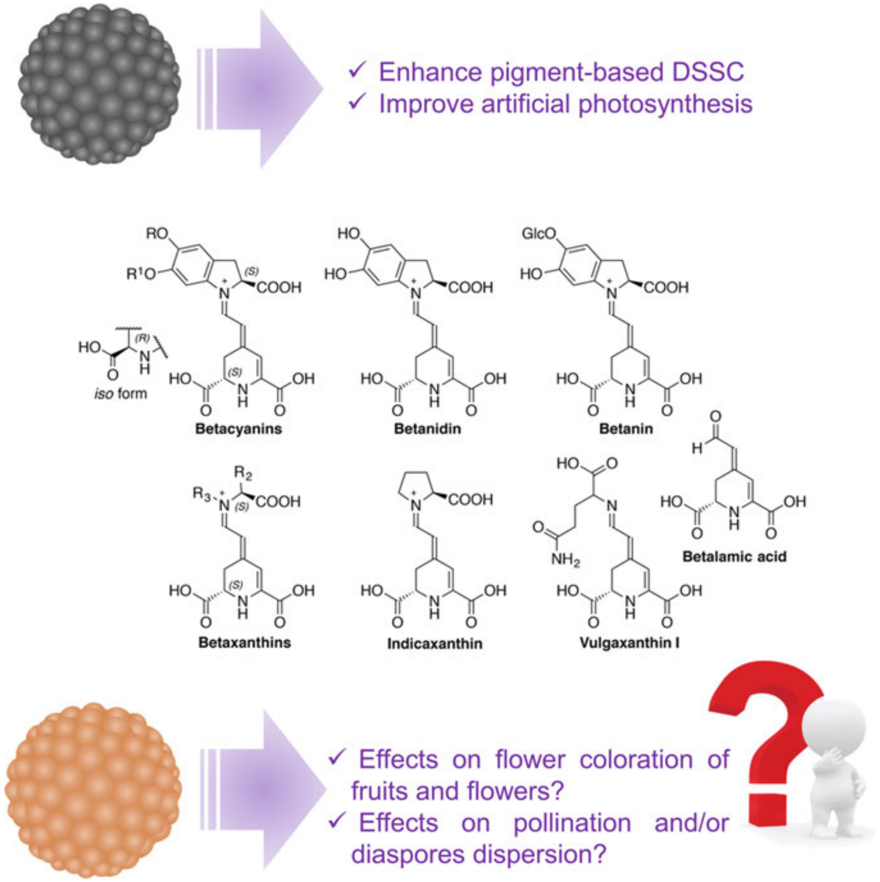


Fig. 5.7 Generic structures of betacyanins and betaxanthins, their biosynthetic precursor betalamic acid and several examples of naturally occurring betalains. Potential positive applications and negative side effects of the interaction of nanomaterials with betalains. (Figure constructed by the authors)

5.6 Optical and Spectroscopic Behavior of Nanomaterials-Pigment Interaction

Electromagnetic radiation is usually divided into γ-rays, x-rays (Roentgen waves), ultraviolet light, visible light, infrared, microwaves, and radio waves. All of these forms propagate as transverse electromagnetic waves with the same speed (speed of light, $c = 2.99792458 \times 10^8\ \mathrm{ms}^{-1}$) in a vacuum (Solè et al., 2005). The differences observed in the electromagnetic spectrum are related to wavelength and frequency (Skoog et al. 1996; Solé et al. 2005). Monochromatic electromagnetic radiation is commonly labeled by interrelated magnitudes: frequency (ν), wavelength (λ), wavenumber ($\bar{\upsilon}$), and energy of the photon (E), correlated as follows:

$$E = \frac{h}{v}$$

where h is Planck's constant (6.62×10^{-34} J.s).

In terms of wavelength and wavenumber.

$$E = \frac{h}{\lambda} = \mathrm{hc}\bar{\upsilon}$$

where h is Planck's constant (6.62×10^{-34} J.s).

The wavenumber as well as the frequency is directly proportional to the energy. Electromagnetic radiation is made up of packets of energy called "photons" or "quanta." During interactions between radiation and matter, molecules in the lowest energy state (ground state) can have this condition changed commonly by absorption, transmission, and/or reflection of part of the absorbed radiation (Lehmann 2016).

The optical or spectroscopic properties of plant pigments during their interaction with ENMs depend on the arrangement between the nanostructure and the pigment molecule. A wide range of analytical methods is used to study the nanoparticle–pigment interaction behavior, particularly with optical interactions. These techniques require low cost and easy management, and fewer time-consuming processes (Lehmann 2016; Santos et al. 2021, 2022; Souza et al. 2022).

Absorption and emission behavior have been reported when plant pigment interacts with NPs because these pigments act as chromophores. For instance, AuNPs with sizes of 5 nm, 10 nm, and 20 nm induce a fluorescence quenching behavior in chlorophyll pigments (Falco et al. 2011). These effects were also related to the decrease in NPs size. These phenomena are correlated to photo-induced electron transfer from excited pigment molecules to the nanoparticle's surface. Similar results are observed with AgNPs by Queiroz et al. (2016). In this way, plasmonically generated changes in the absorption and emission behavior of pigments are reported due to the unique optical properties of metal NPs. These properties can be tuned by modifications of the size, shape, and chemical composition of the metal NPs. In another study, Brecht and co-authors (Brecht et al. 2012) used single-molecule spectroscopy to investigate the plasmonic interaction effects of nanometer-sized hexagonal arrays of Au- and Ag-triangles on the fluorescence properties of chlorophyll pigments onto Photosystem I (PSI), a key component of the photosynthetic apparatus. These studies suggest the potential photochemical and photophysical impact of some NMS on light-harnessing photosynthetic events.

Vibrational spectroscopic methods are also useful to unravel the vibrational modes and/or functional groups of complex chemical species (Farber et al. 2019; Beć et al. 2020). Especially, Fourier transform infrared spectroscopy (FTIR) utilizes the interaction of matter with electromagnetic radiation at the spectral region of 4000–400 cm^{-1} (2500–25,000 nm). Infrared spectroscopy enables excitation of vibrational or vibrational/rotational transitions of molecules involving transitions from rotational and/or vibrational levels in the same ground electronic state (Avran and Meteescu 1972; Stuart et al. 1996) and can be used to study the fundamental vibrations of pigments during their interaction with NMs (Pontes et al. 2019, 2020). In this respect, vibrational spectroscopy is a powerful and accessible optical technique for environmental monitoring; it is a highly accurate, simple method and requires no sample pre-treatment for measurements (Santos et al. 2022). Additionally, recent studies have shown the possibility of developing a fast and accurate discrimination method for changes on molecular vibrational modes of functional groups by its association with machine learning (ML) algorithms (Larios et al. 2020; Oliveira et al. 2021).

5.7 Electrochemical Behavior of Nanomaterials-Pigment Interaction

The use of solar cells offers great advantages because they take advantage of sunlight, which is an inexhaustible resource and available all over the planet. In addition, they are easy to maintain and install and can be installed in places with difficult access. Currently, commercialized solar cells are predominantly based on monocrystalline or polycrystalline silicon crystals that are produced under optimized temperature and pressure conditions using Czochralski's methodology.

Through the photovoltaic effect, solar cells convert sunlight into electrical energy. Dye-sensitized solar cells (DSSCs) are a promising replacement for traditional silicon solar cells (Fig. 5.8). DSSCs are photoelectrochemical cells belonging to the group of hybrid solar cells as they are formed by organic and inorganic materials. A DSSC has a counter electrode and a photoelectrode, which are often deposited on a glass substrate containing a transparent conductive oxide, such as indium-doped tin oxide or fluorine-doped tin oxide. A mesoporous semiconductor layer, usually TiO_2, is deposited on the conductive surface. In the photoanode, dye molecules for sensitization are attached to TiO_2, and photoexcitation occurs when the dye absorbs light; the dye is oxidized and electrons are injected into the TiO_2 conduction band. An electrolyte with a redox couple (I^-/$I3^-$) is responsible for restoring the original state of the dye. The circuit ends with the migration of electrons through an external

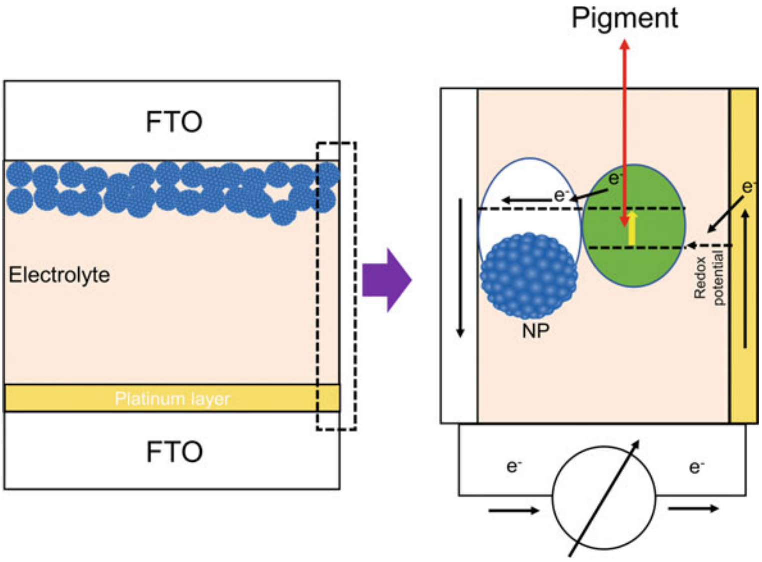


Fig. 5.8 Working principles of DSSC a third generation of solar cell. This type of solar cell was inspired by the photosynthesis process to trap the solar energy that is called as a photon and convert it into electrical energy. FTO: fluorine doped tin oxide electrode; and e^-: electron (Figure constructed by the authors)

charge. DSSCs are attractive and promising because they are mechanically resistant, made of relatively low-cost material, and are very easy to process compared with the silicon solar cells that are currently marketed. They can also have their application expanded because they are lighter and can be produced on flexible substrates (Shalini et al. 2015; Orona-Navar et al. 2021).

As sensitizers for third-generation photovoltaic cells, metal complexes, synthetic or natural dyes are used (Zanan et al. 2016; Orona-Navar et al. 2020). Sensitizers based on metallic and organic dyes, such as ruthenium, achieve good photoconversion efficiency (~11%). However, they have disadvantages related to high toxicity, limited presence in nature, exhaustive processes of synthesis and purification, and high cost (Orona-Navar et al. 2020). An interesting alternative for replacing sensitizers based on synthetic dyes and metal complexes are natural pigments such as chlorophylls, carotenoids, anthocyanins, betalains, tannin flavonoids, and phycocyanin (Ranjitha et al. 2020).

These pigments can be obtained from leaves, fruits, and flowers; recently, pigments from microalgae, fungi, and bacteria have also been studied. The main advantages of applying natural dyes as solar cell sensitizers are their low production costs, simple extraction methods, large-scale production, easy handling, and low to minimal toxicity (Orona-Navar et al. 2020).

Among natural pigments, Chls are naturally suitable for efficient light harvesting. However, their application in photovoltaic cells has a strong limitation: high photodegradation. This is unfortunate because Chls are nature's best photoreceptors. Thus, if the photostability of Chls could be improved, their technological application, especially in photovoltaic cells, would be promising because the long-term stability of the molecules is an indispensable prerequisite for this purpose (Barazzouk et al. 2012). Therefore, combinations of different natural pigments have shown positive results in DSSC sensitization when compared with the use of individual natural pigments. An example of this is the combination of Chls and carotenoids, where Chls fulfill the light-gathering function and carotenoids act as photoprotectors, promoting an improvement in photostability. Furthermore, mixed pigments have also been shown to increase photoconversion efficiency compared with single natural pigments as in the case of the association of Chls and anthocyanins that provide the effect of capturing light in a wider range of the electromagnetic spectrum (Park et al. 2014). However, the photostability of Chls is increased by carotenoids to some extent. Studies show that carotenoids do not protect Chls in vitro as effectively as they photoprotect Chls in vivo. Thus, it is highly imperative to look for an agent that provides efficient in vitro photoprotection of Chls to industrially and biotechnologically enable the use of Chls and other biomolecules as sensitizers for photovoltaic cells (Orona-Navar et al. 2020).

Gold NPs (AuNPs) are described as photoprotective of Chl-*a*, meaning that the photodegradation of Chl-*a* is delayed in the presence of AuNPs. For instance, Barazzouk et al. (2012) have shown that AuNPs cause an increase of up to an order of magnitude in the half-life of Chl-*a*. AuNPs can efficiently bind to the nitrogen sites of Chl, resulting in enhanced protection of these pigments (Mezacasa et al. 2020) and, thus, inhibiting the reaction of reactive oxygen species with Chl-*a*, known to cause its degradation under light. Therefore, under in vitro conditions, AuNPs are much better Chl-*a* photoprotective agents than *ß*-carotene or quinones (Barazzouk et al. 2012). In addition to the photoprotective capacity of Chls, some NPs when incorporated into the photoanode tend to increase the dye's ability to capture photons from sunlight. Among these materials are metallic NPs, such as silver (AgNPs) or AuNPs, among other metals, which, when incorporated into the TiO_2 structure of the photoanode, improve photon absorption. NPs of noble materials added to a photoanode exhibit surface plasmonic resonance within the device, which is the effect of electron oscillation in a structure stimulated by incident light. The solar cell can have embedded Au or Ag NPs to induce the effect, which causes greater absorption and scattering of light and, ultimately, the potential improvement in solar cell performance (Bhogaita and Devaprakasam 2021).

Gold NPs have received special attention due to their electronic and optical properties based on the existence of surface plasmonic resonance. Electrochemical investigations demonstrate that AuNPs on a nanotemplate structure act as an electrical relay (Barazzouk and Hotchandani 2004). Gold NPs are widely used to produce functional electrical coatings; these NMs contribute to improving the redox activity in electrochemical and photochemical applications by easily interacting with organic molecules as in the case of dyes based on biomolecules (Mezacasa et al. 2020). Furthermore, AuNPs can provide better performance in photovoltaic cells due to their potential to accept and transport the photogenerated electrons in Chl-*a* to the collector electrode (Barazzouk and Hotchandani 2004).

5.8 Computer Aided Simulation for Understanding Nanomaterials-Pigment Interaction

The computational method must be chosen according to your goals, i.e., getting the properties of atomic and molecular bindings, such as intra- and intermolecular energies or conformational positions adopted by NP-pigments, for example. It is certainly important to approach your system before any modeling is begun because computational methods are frequently limited to the number of atoms and/or level of complexity of the studied system. Actually, the available processing power is frequently limited, but not the method specifically. This is commonly known as "computational cost," *i.e.*, the number of required processors to calculate the target variables. From this point of view, a simple exercise of imagination can realize the challenge associated with NPs systems modeling for many atoms.

To optimize the simulation study, the choice of ideal method is important because it has resolution dependence; for instance, calculations can be achieved just to evaluate the disposition and conformation of a pigment on the nanomaterial surface by classical methods or details for atom–atom interactions, including chemical reactions, can be computed from quantum approaches. Nevertheless, it is common to merge the classical and quantum methods in a Quantum Mechanics/Molecular Mechanics (QM/MM) model or to perform two different simulation methods separately. For instance, the best conformation adopted for a target macromolecule (NPs or proteins) with the ligand (pigments) is initially computed by a classical method followed by a quantum calculus to reach some electronic property locally.

There are some important computational methods available to study the interactions of NMs with molecules as detailed by A.S. Barnard for inorganic NPs (Barnard 2010). The in silico methods can be separated basically into two categories: empirical and physics methods (Raunio et al. 2015) as shown in Fig. 5.9. Two important empirical methods are the nano-QSAR and Docking. These methods frequently have an intrinsic theoretical background with quantum and/or molecular mechanics incorporated into them (Santiago et al. 2020).

Nano-QSAR is based on a quantitative structure–activity relationship and is applied to predict biological responses, especially toxicity, based upon the physicochemical properties of NMs available in a set of databases (Puzyn et al. 2009). However, to study the interactions at the molecular level, the docking method is a good beginning. In this case, many strategies have been developed to calculate the best poses for the ligand–target as well as to get its score functions, including genetic algorithmic, fragment-based methods, point complementarity, and distance geometry, among others (Taylor et al. 2002). Molecular docking is widely applied to study protein–ligand systems, but some programs and web platforms have been

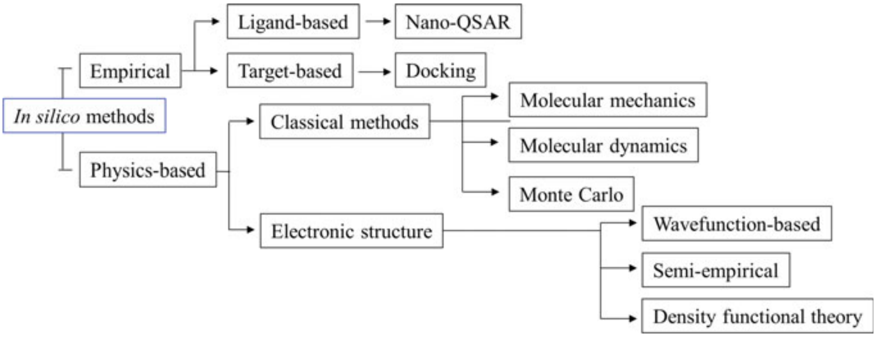


Fig. 5.9 Some computational methods available to study the interactions of pigments and any plant source molecule with macromolecules such as nanoparticles (Figure constructed by the authors)

developed to predict the interactions with the NP surface. An example is the docking calculation performed for the AgNP-xylanase protein (Mishra et al. 2021) using the PatchDock server (Schneidman-Duhovny et al. 2005), a geometry-based molecular docking algorithm program that is a good approach for molecular shape complementarity. Similarly, Fig. 5.10 shows the interaction between the same AgNP of 4.5 nm diameter with *β*-carotene.

From a predicted conformation adopted by the pigments on the surface of the NMs in the docking calculations, more details for specific interactions in time can be achieved by molecular dynamics. This method is based on the resolution of classical equations of motion; Newton's laws of motion implemented into a numerical

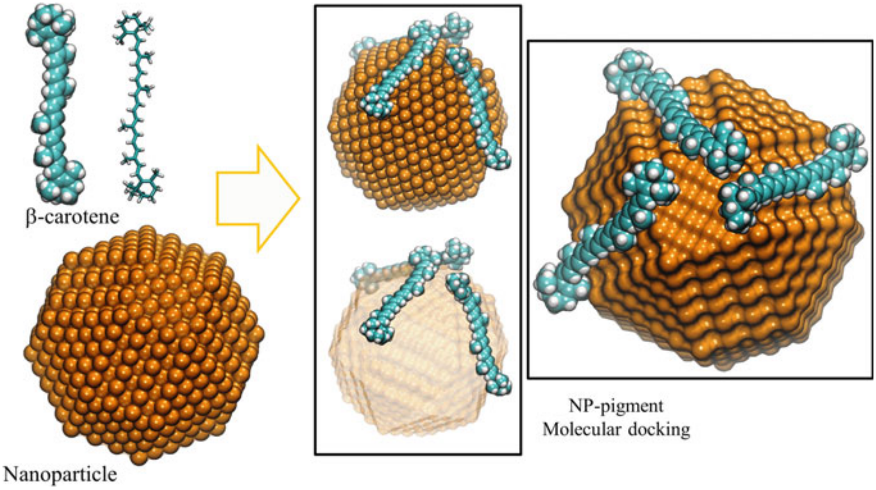


Fig. 5.10 Three conformations were predicted by molecular docking for the AgNP-carotene pigment interaction using PatchDock server for a 4.5 nm diameter sphere (Schneidman-Duhovny et al. 2005). (Figure constructed by the authors)

algorithm, e.g., the Verlet or its variations. Initially, some velocities are attributed to each atom according to the Boltzmann distribution. Then, with a set data of force field parameters for all molecules, the potential interactions are calculated to get the force, which is then used to obtain the acceleration, velocities, and new position coordinates according to a determined time step of integration, commonly one or two picoseconds. These calculations are computed repeatedly to generate a correlated trajectory (Boldon et al. 2015), an advantage in comparison to traditional molecular mechanics or Monte Carlo simulations.

Sometimes, obtaining electronic data for an NP–pigment interaction is desired, such as changes in the response of the radiation interactions and/or electron/energy transfer. For this purpose, an electronic calculation is mandatory. Several methods are available in a set of well-established programs, which are wave function-, semi-empirical-, density functional theory-based, or some derivation thereof (Barnard 2010). These methods are very powerful to investigate interactions at the atom–atom level; however, they are frequently limited to the size of the system due to the high computational cost. Therefore, the quantum approach is applied to a part of the overall system. To illustrate an application of this method, Fig. 5.11 shows an interaction of chlorophyll and pheophytin with gold-NPs performed by our research group (Mezacasa et al. 2020). In this case, to understand the photophysical behavior of these photosynthetic pigments in the presence of Au-NPs, the density functional theory (DFT) was applied. The energies of adsorption were computed as well as the Mulliken charge distribution. The alterations in the absorption and fluorescence profiles of chlorophylls in the presence of the nanomaterial could be associated with the presence of the element magnesium in the structure. The method allows an explanation for the electronic transitions via HOMO–LUMO transfer charges to be suggested.

5.9 Conclusion and Prospects

Current research on the interaction of NMs and plant pigments has opened a new way to view the environmental impact of these materials, and the development of novel technological products. However, ENMs, depending on their dose and physicochemical properties. may represent a potential risk for plant development and environmental health. The nanomaterial–pigment interaction may change the operation of physiological processes such as the chemical energy production of photosynthesis or flower pollination that involves color-guided pollinators. In this scenario, investigations regarding the interactive effects of NPs on non-foliar plant tissues are still required. Finally, the study of novel nano-enabled materials is essential to assess their potentialities, interaction with the environment, and their effects on other plant organisms; hence, further research on plant pigments is necessary.

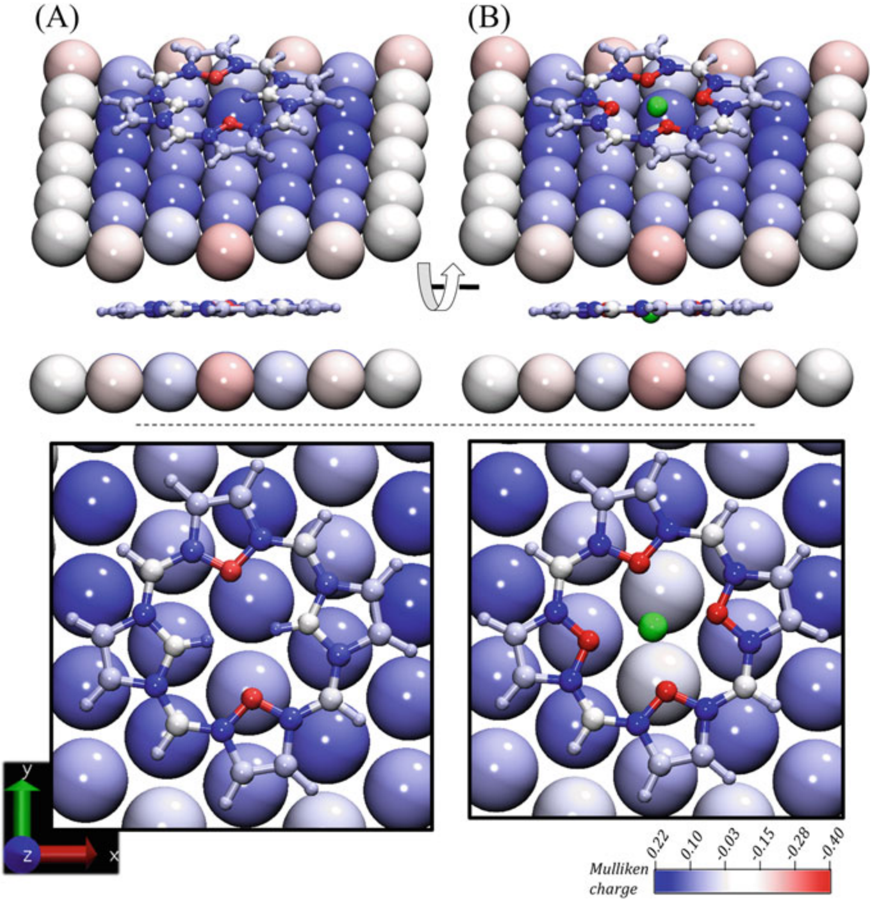


Fig. 5.11 Mulliken charges mapped onto the total electron density of the **A** porphyrin and **B** porphyrin-Mg on gold nanoparticle surfaces. (Figure constructed by the authors)

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Chapter 6
Impact of Nanomaterials on Plant Secondary Metabolism

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Abstract Plants encounter various nanomaterials (NMs) as pesticides and fertilizers. It is also possible that nanomaterials reach plants as waste from consumer products and industry. The effects of such NMs on plants have been widely studied, and both positive and negative effects of NMs on plant growth and development have been reported. Recent metabolomics studies suggest that nanoparticles affect the concentration of secondary metabolites in plants by modulating reactive nitrogen/oxygen species, gene expression, and signaling pathways. Secondary metabolites are plant compounds that accumulate in plants through their secondary metabolism. To date, more than 200,000 defined structures of secondary metabolites have been identified, among which many of them possess antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective, antidepressant, antioxidant, neuroprotective, and anticancer properties. The application of elicitors is a simple strategy to increase the production of secondary metabolites in plant cell and tissues. The ability of nanomaterials to induce plant secondary metabolism has recently been exploited in the elicitation of pharmaceutically important compounds from various plant species. The ability of different NMs to induce the accumulation of different classes of compounds in the same plant species has also been accomplished. The molecular mechanisms behind the effects of NMs on plant secondary metabolism revealed the putative genes involved in NM-mediated elicitation of various plant compounds in several reports. This chapter reviews the current understanding of the effects of nanoparticles on plant secondary metabolism and the elicitation of pharmacologically important compounds from plant species.

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6.1 Introduction

In this era of nanotechnology, nanomaterials (NMs) are finding applications in various fields, including science, industry, medicine, and agriculture. Several consumer products, medicines, fertilizers, pesticides, cosmetics, food packings, paints and electronics containing NMs are already on the market.

Plants are exposed to NMs through various pathways. NMs can move to plants as NM-containing wastes that are released into the environment by industries and consumer products in water and soil. The predicted concentration of some nanoparticles (NPs) in soil is: silver (Ag): 0.91–1.8 ng/kg; titanium oxide (TiO_2): 0.09–0.24 µg/kg; zinc oxide (ZnO): 0.01–0.03 µg/kg (Sun et al. 2014). On the other hand, recent advances in agriculture use formulations containing NMs such as fertilizers, fungicides and pesticides. The concentration-dependent response to NPs varies greatly among different plant species, which has been reported, for example, for NPs from ZnO: 40–1200 ppm, (Mosquera-Sánchez et al. 2020; Sadak and Bakry 2020) cerium oxide (CeO_2): 125–500 ppm, (Rico et al. 2014) copper oxide (CuO): 200–400 ppm, (Wang et al. 2019) and gold (Au): 5 ppm (Kang et al. 2016) to show the effect of nanofertilizers and pesticides. Effective concentrations for plant protection applications range from 2 to 2000 ppm for Ag NPs alone across plant species and pathogens (Elmer and White 2018). Thus, while the application of nanotechnology is expected to revolutionize agriculture, NMs that enter the environment directly as agrochemicals or indirectly as industrial or household wastes are proving to be pollutants with unknown consequences for plants.

Previous studies on various model plant species and crops have shown that NMs affect plant growth and development both positively and negatively depending on their concentration. However, it is known that the biologically relevant concentration of NMs strongly depends on their metallic core, physicochemical properties, substrate and plant species. NMs are known to interfere with metabolic processes and lead to the formation of reactive oxygen species (ROS)/reactive nitrogen species (RNS), damage the structure and function of cell membranes, and reduce enzyme activities and DNA synthesis. Recent literature also suggests that plant secondary metabolism is also affected by NMs.

Secondary metabolism is crucial for plants as they play an indispensable role in plant survival: as protection against herbivores and pathogenic microbes, as signals for symbiotic interactions of plants with beneficial microorganisms, as allelopathic agents in natural habitats for protection against competitors, as physical and chemical barriers against abiotic stressors such as UV radiation, and as endogenous regulators of plant growth regulators.

The small molecular products that are biosynthesized in plants through their secondary metabolic pathways are called plant secondary metabolites. These compounds are generally classified as terpenes, steroids, phenols, flavonoids and alkaloids and are derived from primary metabolites or as an intermediate in the primary metabolic pathway (Chandran et al. 2020; Pang et al. 2021). Plant secondary metabolites play an important role in plant defense mechanisms against biotic and abiotic stresses (Khare et al. 2020; Mahajan et al. 2020). In particular, phenylpropanoids are involved in the regulation of oxidative stress, free ion chelation, cell wall lignification, and plant defense (Agati et al. 2012). In addition, secondary metabolites are also known to be involved in pest defense (Barlow et al. 2017; Stevenson 2020), signal transduction in plant–microbe symbiosis (Adedeji and Babalola 2020) and plant innate immunity (Piasecka et al. 2015).

Apart from their beneficial effects in plants, many secondary metabolites are economically important as medicines, flavors and fragrances, dyes and pigments, pesticides and food additives. Useful remedies from herbal medicine are due to the presence of various secondary metabolites (Chandran et al. 2020). For example, a recent study showed that 12 pure compounds from *Clerodendranthus spicatus* (Thunb.) C. Y. Wu ex H. W. Li, an herb widely used in traditional Chinese medicine for the treatment of kidney inflammation, gout, and dysuria, promoted the excretion of uric acid (Chen et al. 2020). More than 500 secondary metabolites have been reported from 46 species of the genus *Lycopodium*, and these secondary metabolites have been shown to have several medically important bioactivities, including neuroprotective, anti-inflammatory, anti-cancer, antiviral, and antimicrobial activities (Wang et al. 2021).

The quantity of secondary metabolites produced by natural biosynthesis in plants is limited to meet the growing demand of the pharmaceutical industry. Thus, development of alternative biotechnological approaches is necessary to boost production of secondary metabolites (Thakur et al. 2019). Elicitation is one of the most commonly used techniques to enhance the biosynthesis of secondary metabolites (Thakur et al. 2019; Yazdanian et al. 2021).

In recent years, NMs have emerged as novel triggers for inducing biosynthesis of bioactive compounds in plants (Shakya et al. 2019; Rivero-Montejo et al. 2021). Ag NP treatment increased artemisinin content by 3.9-fold in 20-day-old hairy root cultures of *Artemisia annua* L. (Zhang et al. 2013). Hydroponically grown *Bacopa monnieri* L. treated with copper-based NPs (Cu) improved antioxidant capacity and showed hormetic increase in the content of saponins, alkaloids, flavonoids and phenols (Lala 2020). Celastrol, a therapeutically important phytochemical, was increased in adventitious and hairy root cultures of *Celastrus paniculatus* Willd. after treatment with Ag NP (Moola et al. 2021). The elicitation of various classes of bioactive secondary metabolites in *Hypericum perforatum* L. cell suspension cultures treated with various metal (Ag, Au, Cu, Pd) and metal oxide (CeO_2, CuO, TiO_2, ZnO) NPs has been recently reported (Kruszka et al. 2022).

In this chapter, we discuss the effects of NMs on secondary metabolism in plants, focusing on signaling events and key medicinal agents that are enhanced by NPs.

6.2 Plant's Response to Nanomaterials

Exposure to NMs has been found to induce changes in various physiological, morphological and developmental processes of plants. In general, plant metabolism can be divided into primary (associated with energy and biosynthesis of building blocks) and secondary (more specialized molecules) metabolism (Erb and Kliebenstein 2020). Primary metabolites consist of the products of photosynthesis, glycolysis, the tricarboxylic acid cycle (TCA cycle), biosynthesis of amino acids, lipids, and some natural polymers. Cu NPs minimized the negative effects of drought stress on photosynthetic pigments and promoted plant growth, development and grain yield in *Zea mays* L. (Van Nguyen et al. 2021). Foliar application of silica (SiO_2) and ZnO NPs in *Cucumis sativus* L. significantly increased chlorophyll content and various amino acids and modulated carbon metabolic processes in leaves (Li et al. 2021a). In contrast to primary metabolism, secondary metabolism yields structurally diverse and specialized metabolites, such as phenylpropanoids (polyphenols, flavonoids, anthocyanins, xanthones, stilbenes), terpenes, polyketides, prenylated phloroglucinols, alkaloids, and organosulfur compounds (glucosinolates, thioesters). These metabolites play a role as phytoalexins, phytoanticides and phytoncides (defense systems against many biotic stresses), antioxidants (control ROS), chelators (scavenging free metal ions), UV protectants, growth regulators, and factors against abiotic stresses (Feng et al. 2021b; Nobahar et al. 2021). Various NPs including iron (Fe), cerium (Ce), and SiO_2, altered secondary metabolite content in lettuce and pepper seedlings (Kalisz et al. 2021).

6.2.1 *Impact of NPs on Precursors of Secondary Metabolism*

The effects of different types of NMs on precursors of secondary metabolites have been analyzed in detail in algal, monocotyledonous, and dicotyledonous plant models (Table 6.1). Many studies have captured the effects of NMs on the pentose phosphate pathway, glycolysis, and the TCA cycle, and have linked carbohydrates and organic acids to these processes. The upregulation of these metabolic pathways and compounds is related to defense mechanisms and their additional roles as chelators and osmoprotectors (Li et al. 2019; Nobahar et al. 2021). Moreover, Ag (Chavez Soria et al. 2017), CuO (Zhao et al. 2017a), $Cu(OH)_2$ (Zhao et al. 2018a), CdO (Večeřová et al. 2016), CeO_2 (Salehi et al. 2018), graphene-based (Hu and Zhou 2015; Ouyang et al. 2015; Chen et al. 2021), WS_2 (Yuan et al. 2018) and fullerols (Zhao et al. 2019) affected the fatty acids and lipid compositions of various plant species such as *Arabidopsis thaliana* (L.) Heynh., *C. sativus, Z. mays, Hordeum vulgare* L. and *Phaseolus vulgaris* L.

Amino acid metabolism is an important bridge between primary and secondary metabolites. Many amino acids are important precursors in the biosynthesis of alkaloids (e.g., arginine, lysine, ornithine, phenylalanine, proline, tryptophan, tyrosine),

Table 6.1 Alteration of plant primary metabolites due to NP treatment/exposure

Plant species	Treatment (NPs, variants, size, concentration)	Omic approach	Changes in metabolism	Reference
Arabidopsis thaliana (L.) Heynh	Citrate-Ag 1–10 nm, PVP-Ag 1–10 nm, Ag^+ ($AgNO_3$), 1 mg/L	M	↑N-acylethanolamines, ↑ phytosphingosine (d20:3, d20:2), ↓ purine nucleoside (PVP-Ag), ↓fatty acids, ↓ lyso-PG, ↓ lyso-PE, ↓ lyso-PC	Chavez Soria et al. (2017)
	Ag 10 nm, Ag^+ ($AgNO_3$), 12.5 mg/L	M	↑TCA cycle, ↑ carbohydrates, ↑ threonine, ↓ amino acids (Val, Ser and Asp, melatonin)	Ke et al. (2018)
	PVP-AgNPs 32 nm, 30 mg/L	T	Trp metabolism, 2-oxocarboxylic acid metabolism, α-linolenic acid, Gly, Ser and Thr metabolism	Zhang et al. (2019a)
	CuO > 50 nm, 10 mg/L	M	↑ amino acids (N-acetylornithine, Phe)	Chavez Soria et al. (2019)
	ZnO 20 nm, 0.16, 0.8, 4, 20, 100 mg/L	T	pyruvate decarboxylase-2, glutathione transferase, fructan exohydrolase,	Landa et al. (2015)
Cucumis sativus L	C60 Fullerols 1, 2, 5 mg/plant	M	↓ fatty acids (linolenic acid)	Zhao et al. (2019)
		P	↑chloroplast proteins (PSII, CAB, Mg-PPIX, Cyt b6f), ↑ glycolysis proteins, ↑ antioxidant proteins (ferritin, cystatins, tocopherol cyclase), ↓ TCA-cycle proteins, ↓ GST,	
	Ag 20 nm, 4, 40 mg, Ag^+ ($AgNO_3$), 0.04, 0.4 mg	M	↑ TCA-cycle, ↑carbohydrates and polyols, ↑aminoacids, ↓ N-metabolism,	Zhang et al. (2018)

(continued)

Table 6.1 (continued)

Plant species	Treatment (NPs, variants, size, concentration)	Omic approach	Changes in metabolism	Reference
	CuO 40 nm, 200, 400, 800 mg/kg	M	amino acids (↑ Gly, ↑ Pro, ↓Asp, ↓Cit, ↓Met, ↓Pip, ↓ox-Pro, ↓Orn), carbohydrates (↑ xylose, ↑ fructose), organic acids (↑ glutaric, ↑ lactic acid, ↓citric, ↓xylonic acid), fatty acids (↑ caprylic, ↑ linolenic, ↑ pelargonic acid, ↓capric acid), polyols (↓myo-inosytol)	Zhao et al. (2017a)
	CuO 40 nm, 10, 20 mg/L	M	↑amino acids, ↓ organic acids	Zhao et al. (2016a)
	Cu^{2+} ($CuSO_4$), 0.21, 2.1, 10 mg	M	↑ aromatic amino acids, ↓ TCA-cycle,	Zhao et al. (2018b)
Cucumis sativus L	$Cu(OH)_2$, 50–1000 nm, 2.5, 25 mg	M	↑ polyols, ↑ saturated fatty acids, ↓ carbohydrates (pentose), ↓ unsaturated fatty acids,	Zhao et al. (2018a)
Zea mays L			↑ glycolysis, ↑ TCA-cycle, ↑ carbohydrates, ↑ saturated fatty acids, ↑ amino acids and ↑ N-metabolism, ↓unsaturated fatty acids	
Oryza sativa L	TiO_2 20 nm, 100, 250, 500 mg/L	M	↑ glycolysis, ↑ pentose phosphate metabolism, ↑ TCA-cycle, ↓ glyoxylate and ↓dicarboxylate metabolism	Wu et al. (2017)
	ZnO 30 nm, 0–100 mg/L	M	Ala, Asp, Glu metabolism, taurine and hypotaurine metabolism	Li et al. (2021b)

(continued)

Table 6.1 (continued)

Plant species	Treatment (NPs, variants, size, concentration)	Omic approach	Changes in metabolism	Reference
	GO 0.5–5 µm × 2.0 nm, 0.01–1.0 mg/L	M	↓ galactose metabolism, ↓ glyoxylate and ↓ dicarboxylate metabolism, ↓ TCA-cycle, ↓ amino acids metabolism (Iso, Leu, Val, Gly, Ser, Thr)	Li et al. (2018a)
		P	↓ galactose metabolism, ↓pentose phosphate pathway, ↓ starch and sucrose metabolism, ↓ sulfur metabolism, ↓ glycolysis, ↓ amino sugar and ↓ nucleotide	
Hordeum vulgare L	CdO 7–60 nm, 2.03×10^5 particles/cm^3	M	↑ biosynthesis of aromatic amino acids, ↑ fatty acids, ↓ carbohydrates, ↓ TCA-cycle,	Večeřová et al. (2016)
Spinacia oleracea L	CeO_2 10–30 nm, 0.3, 3 mg	M	↑ amino acids, ↓ carbohydrates, ↓ organic acids	Zhang et al. (2019b)
Phaseolus vulgaris L	CeO_2 10–30 nm, 250, 500, 1000, 2000 mg/L	M	↓ lipids, ↓ polyols, ↓ carotenoids,	Salehi et al. (2018)
		P	↑ glutamine synthetase, ↑ lipoxygenase, ↑ lipid-transfer protein, ↓ alpha-galactosidase, ↓ inositol monophosphatase	
Lactuca sativa L	$Cu(OH)_2$ 50 nm, 8.75, 12.9, 17.5 mg/pot	M	↑ amino acids, ↓ TCA-cycle	Zhao et al. (2016b)
Solanum lycopersicum L	MWCT 50 mg/L	M	↓ cysteine and methionine and carbon metabolism	McGehee et al. (2017)

(continued)

Table 6.1 (continued)

Plant species	Treatment (NPs, variants, size, concentration)	Omic approach	Changes in metabolism	Reference
Chlorella vulgaris Beijerinck	GOQDs 10–40 nm, 0.1–10 mg/L	T	Exposure: ↑ nitrogen metabolism, ↑ Arg and Pro metabolism, ↑ porphyrin and chlorophyll metabolism, Recovery: ↑ carbon fixation, ↑ glyoxylate and ↑ dicarboxylate metabolism, ↑ propanoate metabolism, ↑ Val, Leu and Ile degradation, ↓ photosynthesis,	Kang et al. (2019)
		M	Exposure: ↑galactose metabolism, ↑ Lys biosynthesis, ↓aminoacyl-tRNA biosynthesis, Phe metabolism, Gly, Ser and Thr metabolism, ↓ Tyr metabolism, ↓ Ala, Asp, Glu metabolism Recovery: ↑ galactose metabolism, ↑ Gly, Ser and Thr metabolism, ↑ Phe metabolism, ↑ starch and sucrose metabolism, Recovery: ↓ methane metabolism	
	GO 0.8 − 1.0 nm, GOQD 4.8 − 5.2 nm, 0.01–10.0 mg/L	M	Amino acids (↑ Ala, ↑ Iso, ↑ Val, ↑ Glu, ↓ Asp, ↓ Ser, ↓ Thr), ↑ fatty acids,	Ouyang et al. (2015)
	Metal-WS_2 (nanosheets), 0.1, 1, 10 mg/L	M	↑ Ala, Asp and Glu metabolism, ↑ Arg and Pro metabolism, ↑ GSH metabolism, ↑ Gly, Ser and Thr metabolism, ↓ glycerolipid metabolism, ↓ starch and sucrose metabolism	Yuan et al. (2018)

(continued)

Table 6.1 (continued)

Plant species	Treatment (NPs, variants, size, concentration)	Omic approach	Changes in metabolism	Reference
Triticum aestivum L	G, GO, 0.4–2.0 μm × 0.8 nm, 200 mg/L	M	↑carbohydrate, ↑ amino acid, ↑ fatty acid, ↓ glycolysis, ↓ BCCA	Hu and Zhou (2015)
	GO, rGO 1 - 5 μm × 0.8–1.2 nm, GOQD 10–50 nm × 2.1–2.5 nm,	M	↑ soluble sugar	Li et al. (2018b)
Withania somnifera (L.) Dunal	Zn:AgO, 20–50 nm, MWNT, 20 mg/L	M	↑ Calvin cycle, ↑ carbohydrate metabolism	Singh et al. (2019)
Medicago sativa L	G 20–70 nm, 1–2%	T	↑ biosynthesis of amino acids, ↑ linoleic acid metabolism	Chen et al. (2021)

Abbreviations: G-graphene, GO-graphene oxide, GOQD-graphene oxide quantum dots, MWNT-multiwalled carbon nanotubes, M-metabolomics, P-proteomics, T-transcriptomics, direction: ↑ up-regulation/increasing, ↓ down-regulation/decreasing

glucosinolates (e.g. methionine, leucine, isoleucine, phenylalanine, tryptophan), and phenylpropanoids (e.g., phenylalanine and tyrosine) (Barros and Dixon 2020; Jan et al. 2021). Ag, CuO, $Cu(OH)_2$ NPs and Ag^+, Cu^{2+} ions stimulated accumulation of aromatic amino acids in *C. sativus* tissues, *Z. mays* (Zhao et al. 2016a, 2018b, 2018a; Zhang et al. 2018), *A. thaliana* (Chavez Soria et al. 2019) and *Triticum aestivum* L. (Feng et al. 2021a). The biosynthesis of other amino acids was up regulated by, ZnO (Li et al. 2021b), C_{60} fullerols (Zhao et al. 2019) and graphene NPs (Chen et al. 2021; Hu and Zhou 2015).

6.2.2 Impact of NPs on Secondary Metabolism

A number of studies reported the effects of NPs on plant secondary metabolism (Table 6.2). Accumulation of shikimate and phenylpropanoid pathway products was observed in cucumber and maize after foliar application of $Cu(OH)_2$ (Zhao et al. 2018a) in wheat exposed to Ag (Feng et al. 2021a), in pepper exposed to SiO_2 or Fe_2O_3 (Kalisz et al. 2021) and in *A.thaliana* exposed to CuO (Chavez Soria et al. 2019) NPs. On the other hand, the amount of phenylpropanoids in lettuce, spinach, cucumber, and barley were decreased by $Cu(OH)_2$ (Zhao et al. 2016b, 2017b), CeO_2 (Zhang et al. 2019b), soil application of CuO (Huang et al. 2019), and CdO (Večeřová et al. 2016). Relatively low doses of CeO_2 NPs induced metabolic reprogramming by affecting flavonoids and phenolic compounds in roots and leaves of *P. vulgaris* (Salehi et al. 2020).

Table 6.2 Alteration of plant secondary metabolites due to NPs treatment/exposure

Plant species	Treatment NPs, variants, size, concentration)	Omic approach	Changes and direction	Reference
Arabidopsis thaliana (L.) Heynh	Ag 10 nm, Ag^+ ($AgNO_3$), 12.5 mg/L	M	↓ shikimate-phenylpropanoid (gallic acid, benzoic, scopoletin)	Ke et al. (2018)
	Ag 10, 40, 100 nm, Ag^+ ($AgNO_3$), 0.5, 1, 5 mg/L	M	↑ indole phytoalexins (camalexins derivatives)	Kruszka et al. (2020)
	PVP-AgNPs 32 nm, 30 mg/L	T	glucosinolate biosynthesis tropane, piperidine and pyridine alkaloid biosynthesis	Zhang et al. (2019a)
	CuO > 50 nm, 10 mg/L	M	↑ phenylpropanoids (p-coumaroylagmatine, scopoletin), ↓ isothiocyanates	Chavez Soria et al. (2019)
Cucumis sativus L.	C60 Fullerols 1, 2, 5 mg/plant	M	↑ shikimate-phenylpropanoids (quinic acid, 3-hydroxyflavon, 4-vinylphenol, 1,2,4-benzenetriol, methyl trans-cinnamate),	Zhao et al. (2019)
	Ag 20 nm, 4, 40 mg,	M	↑ shikimate-phenylpropanoids	Zhang et al. (2018)
	Ag^+ ($AgNO_3$), 0.04, 0.4 mg		↑ shikimate-phenylpropanoids	
	CuO 40 nm, 400, 800 mg/kg	M	↑ benzoates (gallic acid, benzoic acid), ↓ phenylpropanoids (o-, p-coumaric, caffeic, ferulic, chlorogenic acid), vanillic acid, dehydroascorbic acid, gluthatione, curcumin, α-tocopherol	Huang et al. (2019)
	CuO 40 nm, 200, 400, 800 mg/kg	M	↑ benzoic acid	Zhao et al. (2017a)
	CuO 40 nm, 10, 20 mg/L	M	↑ shikimate-phenylpropanoids	Zhao et al. (2016a)
	Cu^{2+} ($CuSO_4$), 0.21, 2.1, 10 mg	M	↑ phenylpropanoids, ↑ anthocyanins, ↓ flavonoids, ↓ ascorbate	Zhao et al. (2018b)
Cucumis sativus L.	$Cu(OH)_2$, 50–1000 nm, 2.5, 25 mg	M	↑ shikimate-phenylpropanoids	Zhao et al. (2018a)
Zea mays L.			↑ shikimate-phenylpropanoids	

(continued)

Table 6.2 (continued)

Plant species	Treatment NPs, variants, size, concentration)	Omic approach	Changes and direction	Reference
Oryza sativa L.	TiO_2 20 nm, 100, 250, 500 mg/L	M	↑ shikimate metabolism	Wu et al. (2017)
	ZnO 30 nm, 0–100 mg/L	M	phenylpropanoid biosynthesis	Li et al. (2021b)
	GO 0.5 – 5 μm × 2.0 nm, 0.01–1.0 mg/L	P	↓ phenylpropanoids metabolism	Li et al. (2018a)
Hordeum vulgare L.	CdO 7–60 nm, 2.03×10^5 particles/cm^3	M	↓ phenolic compounds	Večeřová et al. (2016)
Hpericum perforatum L.	Ag 15 nm, Au 14 nm, Cu 25 nm, Pd 15 nm 25 mg/L	M	↑ xanthones, benzophenones, benzoates, anthraquinones ↓ flavonoids, hydroxycynamic acid derivatives	Kruszka et al. (2022)
	CeO_2 10 nm, CuO 25–55 nm, TiO_2 5–15 nm, ZnO 30–40 nm 25 mg/L		↑ flavonoids, xanthones	
Spinacia oleracea L.	CeO_2 10–30 nm	M	↓ phenolics	Zhang et al. (2019b)
	$Cu(OH)_2$ 50 nm, 1.8, 18 mg/L, Cu^{2+} ($CuSO_4$), 0.15, 1.5 mg/L	M	↓shikimate-phenylpropanoids (ferulic acid), ↓ antioxidants (ascorbic acid, threonic acid, tocopherol)	Zhao et al. (2017b)
Phaseolus vulgaris L.	CeO_2 10–30 nm, 0, 250, 500, 1000, 2000 mg/L	M	↑shikimate-phenylpropanoids (cinnamyl acetate, salicin, lignin), ↑ flavonoids and isoflavonoids, ↑↓terpenes, ↑↓ alkaloids	Salehi et al. (2018)
Lactuca sativa L.	$Cu(OH)_2$ 50 nm, 8.75, 12.9, 17.5 mg/pot	M	↓ shikimate-phenylpropanoids (caffeic acid, chlorogenic acid, 3,4-dihydroxycinnamic acid),	Zhao et al. (2016b)
Lactuca sativa L.	CeO_2 4 nm, Fe_2O_3 6 nm, and SiO_2 10 nm, 1.5% suspension	M	↓ 3,4-diOH-benzaldehyde, ↓ ferulic acid, ↓ p-coumaric acid, ↓ salicylic acid, ↓vanillin, ↑ gallic acid, ↑ vanillic acid	Kalisz et al. (2021)
Capsicum annuum L.		M	↑ chlorogenic acid, ↑ neochlorogenic acid, ↑ ferulic acid, ↑protocatechuic acid	

(continued)

Table 6.2 (continued)

Plant species	Treatment NPs, variants, size, concentration)	Omic approach	Changes and direction	Reference
Solanum lycopersicum L.	MWCT 50 mg/L	M	↑ anthocyanins, ↑ carotenoids, ↑ diterpenoids↓ isoquinoline alkaloid biosynthesis, ↓flavone and flavanol biosynthesis	McGehee et al. (2017)
Triticum aestivum L.	G, GO, 400–2000 × 0.8 nm, 200 mg/L	M	↑shikimate	Hu and Zhou (2015)
	Ag	T	↑ phenylpropanoid biosynthesis	Feng et al. (2021a)
Withania somnifera (L.) Dunal	Zn:AgO, 20–50 nm, MWNT, 20 mg/L	M	↑ anthocyanins, ↑ terpenoid (withanolide) biosynthesis	Singh et al. (2019)
Medicago sativa L.	G 20–70 nm, 1–2%	T	↑ isoflavonoid biosynthesis, ↑ phenylpropanoid biosynthesis	Chen et al. (2021)

Abbreviations: G-graphene, GO-graphene oxide,MWNT- multiwalled carbon nanotubes, M-metabolomics, P-proteomics, T-transcriptomics, ↑ up-regulation/increasing, ↓ down-regulation/decreasing

The concentration of benzoic acid and gallic acid was increased, while the content of hydroxycinnamic acid derivatives was reduced in *C. sativus* when exposed to CuO NPs (Huang et al. 2019). In *Solanum lycopersicum* L., more anthocyanins and fewer flavonoids were formed after treatment with MWCT (McGehee et al. 2017).

Metal and metal oxide NPs have got impact on the biosynthesis of phenylpropanoids in the *Hypericum perforatum* L. cells (Kruszka et al. 2022). Metal nanoparticles (Ag, Au, Cu and Pd) increased accumulation of xanthones, prenylated xanthones and beznophenones and reduced levels of flavonoids and hydroxycinnamic acid derivatives in cells. In contrast to this, the level of flavonoids was increased in biomass by the CuO nanoparticles treatment.

NMs have altered the metabolism of alkaloids, a group of compounds that possesses high biological value as defense metabolites (Erb and Kliebenstein 2020). Salehi et al. (2018) reported that the concentration of (s)-corytuberine, laudanosine, and precursors of naphthylisoquinoline alkaloids decreased, while the content of demecolcine, β-caconine, and tropionone increased in *P. vulgaris* after foliar application of CeO_2 NPs. Accumulation of taxane and tropane alkaloids was reported after Ag NPs treatment (Shakeran et al. 2015; Jamshidi and Ghanati 2017) and hyoscyamine and scopolamine after exposure to ZnO NPs (Asl et al. 2019). Metabolome and transcriptome analyses have shown that the biosynthesis of isoquinoline alkaloids was downregulated by MWCT in *S. lycopersicum* (McGehee et al. 2017) and GOQD (graphene oxide quantum dots) in *Chlorella vulgaris* Beijerinck (Kang et al. 2019).

Camalexin is a major indole phytoalexin produced by *A. thaliana* in response to biotic and abiotic stresses. Application of Ag NPs induced the accumulation of this compound (Kruszka et al. 2020). Transcriptomic analysis showed that metabolism of tryptophan (camalexin precursor) is upregulated by exposure of *A. thaliana* to PVP-Ag NPs (Zhang et al. 2019a). The same research shows that exposure upregulates the biosynthesis of glucosinolates—precursors of isothiocyanates (biologically active form).

6.3 Molecular Mechanisms of Nanomaterials-Induced Secondary Metabolic Changes

Secondary metabolite profiles in plants are dynamic and can change under biotic (pathogen and insect attack) and abiotic (UV radiation, drought, temperature, salinity and heavy metals.) stress conditions. In particular, the interaction between NMs and plants leads to overproduction of ROS, oxidative stress, membrane structure impairment, alteration of antioxidant activities, altered secondary metabolism, hormone pathways, and signal transduction (Fu et al. 2014; Hossain et al. 2015). For example, secondary metabolic changes were associated with increased levels of ROS, phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) in *A. thaliana* exposed to 250 and 1000 mg/L CeO_2 and indium oxide (In_2O_3) NPs, respectively (Ma et al. 2016). Moreover, they also alter the expression of genes related to cell division, cell organization, electron transport, and biotic and abiotic stress pathways (Landa et al. 2012; Van Aken 2015). The molecular mechanisms associated with changes of secondary metabolites triggered by NMs are summarized in Fig. 6.1.

6.3.1 Reactive Oxygen Species

ROS is the most rapid response of plants to all stresses and plays a dual role in both triggering the defense system and enhancing cell damage or disruption of signal transduction (Dat et al. 2000). NMs are known to induce ROS in plants (Marslin et al. 2017; Ranjan et al. 2021). The induction of ROS has been observed in both apoplast and chloroplast, preceded by intracellular calcium and MAPK signaling mechanisms (Marslin et al. 2017). Moreover, the molecular aspects of NPs-induced ROS on cell wall-related processes and secondary metabolism have been studied in detail for all stimulatory and inhibitory effects in plants (Berni et al. 2019). It was found that the effect of NPs on the plant system is concentration dependent. Higher concentrations were found to be toxic while lower concentrations resulted in beneficial effects (Jalil and Ansari 2019).

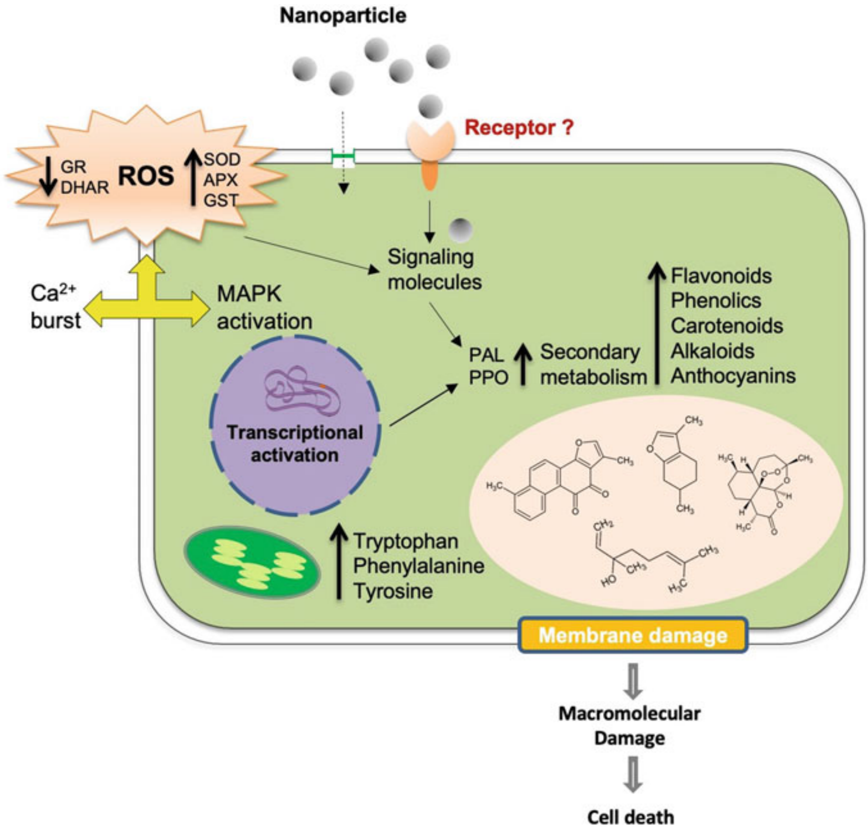

Fig. 6.1 Schematic diagram showing various cellular responses in response to NMs. NMs cause oxidative stress through overproduction of ROS, activation of the antioxidant defense system, lipid peroxidation, membrane damage, calcium bursts, activation of MAPK signaling pathways, and altered secondary metabolism in plants. Upward pointing arrows indicate increased abundance and downward pointing arrows indicate decreased abundance in the plant cell. Abbreviations: SOD, superoxide dismutase; APX, ascorbate peroxidase; GST, glutathione transferase; GR, glutathione reductase; DHAR, dehydroascorbate reductase; PAL, phenylalanine ammonia lyase; PPO, polyphenol oxidase. (Figure constructed by G. Franklin and P. Shakya)

The ROS mechanism triggered by NPs to induce oxidative stress has been studied in different plant systems. In *A. thaliana*, the accumulation of ROS was induced by exogenous application of 100–5000 mg/L Ag NPs. These Ag NPs activate Ca^{2+} and ROS signals by inducing a transient increase in Ca^{2+} and direct oxidation of the major plant antioxidant, L-ascorbic acid (Sosan et al. 2016). In *Allium cepa* L. treatment with 0–80 mg/L Ag NPs led to the formation of ROS, resulting in DNA structural damage and eventual cell death (Panda et al. 2011). Treatment with Ag NPs altered proteins involved in redox regulation and sulfur metabolism in *Eruca sativa* Mill. roots (Vannini et al. 2013). Moreover, the formation of ROS was observed in *Spirodela polyrhiza* L. by inhibiting ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and photoprotective capacity of PSII in the presence of Ag NPs (Jiang et al. 2017). Another study demonstrated the role of NiO NPs in *H. vulgare* by reporting that the overproduction of ROS led to oxidative stress and increased lipid peroxidation. However, simultaneous treatment of SiO_2 NP with NiO resulted in an antioxidant response with decreased lipid peroxidation, highlighting the protective role of nano-SiO_2 (Soares et al. 2018). Another study shows that the phytotoxic potential of cobalt oxide (Co_3O_4) NPs reduces seed germination, root growth, DNA and mitochondrial damage, oxidative stress and cell death in eggplant, while it increases ROS, membrane potential and nitric oxide (NO) (Faisal et al. 2016). Besides generating ROS, Ag and Ag^+ NPs coated with polyvinylpyrrolidone (PVP) promote gene expression of stress-related genes in *A. thaliana* (Kaveh et al. 2013).

6.3.2 Calcium Ion Signaling

During various stresses, Ca^{2+} ions act as second messengers and provide Ca^{2+} ion channels for plant adaptation to adverse conditions (Tuteja and Mahajan 2007). The interaction of fullerene C_{60} nanocrystals (nano-C60) suspended in water with Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) has been shown to modulate Ca^{2+} signal transduction function (Miao et al. 2014). In addition, Ag NPs bind to calcium receptors, Ca^{2+} ion channels, and calcium-sodium ATP pumps, thereby regulating cell metabolism. Ag NPs in *Oryza sativa* L. have been found to be involved in Ca^{2+} ion regulation and signaling, protein degradation, cell wall synthesis, transcription, oxidative stress tolerance, cell division, and apoptosis (Mirzajani et al. 2014). Proteomic studies also revealed the abundance of superoxide dismutase (SOD), L-ascorbate peroxidase (APX) and glutathione transferase (GST) in detoxification or oxidative reaction pathway (Mirzajani et al. 2014). Reports showed the role of NO in increasing cytosolic Ca^{2+} ions using *Nicotiana plumbaginifolia* L. cells and it also stimulates the activity of protein kinases during physiological processes (Lamotte et al. 2006).

6.3.3 Phytohormone Signaling

Plant metabolism is highly influenced by hormone regulation during plant growth, which mediates numerous responses to plant stresses (Santner et al. 2009). Several reports have shown the significant influence of NPs on plant hormones. For example, Fe_2O_3 NP uptake had a significant effect on IAA and ABA content in roots of transgenic and non-transgenic rice (Gui et al. 2015). Similarly, CeO_2 NPs have a differential effect on indole-3-acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA) in leaves and roots of transgenic and conventional Bt cotton compared to the control group (Nhan et al. 2015). Thin-walled carbon nanotubes (CNTs) treatment reduced the growth of *O. sativa* seedlings by decreasing the content of endogenous plant hormones such as IAA, GA, IPA, JA, BR and ABA (Hao et al. 2016). In *A. thaliana*, the response to ZnO NPs is associated with a decrease in growth, cytokinins and auxins in apices. Moreover, a higher dose led to an increase in the levels of ABA and SA, while it suppressed the levels of JA (Vankova et al. 2017). Similarly, Ag NPs were found to inhibit ethylene perception (ET) by hindering ET biosynthesis in *A. thaliana* (Syu et al. 2014).

6.3.4 Nitric Oxide (NO) Signaling

NO is a universal signaling molecule that plays an important role in nanomaterial-triggered changes in plant secondary metabolism. For example, NO burst leads to the accumulation of saponins and artemisinin during fungal attack (Zhang et al. 2012). In *Pisum sativum* L., NO showed protection against Ag NP induced phytotoxicity through increased superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity and reduced glutathione reductase (GR) and dehydroascorbate reductase (DHAR) activities (Tripathi et al. 2017). On the other hand, CdO NPs showed a significant effect on primary metabolism of barley plants with an increase in total amino acids in roots and leaves and a decrease in saccharides in roots, but had no effect on secondary metabolites (Večeřová et al. 2016).

6.4 Applications of Nanomaterial-Induced Secondary Metabolic Changes

6.4.1 NPs as Biostimulants

Plant biostimulation is a process that leads to changes in plant metabolism in order to use available environmental resources more efficiently, increase tolerance to environmental stresses, and increase yield (Juárez-Maldonado et al. 2019). NPs are used as novel biostimulants to promote plant growth under stress conditions. Stimulation of secondary metabolites such as alkaloids, terpenoids, phenolic compounds, glucosinolates and flavonoids reduces the deleterious effects of environmental stress in plants (Rajput et al. 2021). For example, increased melatonin synthesis by application of ZnO NPs helped in controlling drought-induced damage in *Z. mays* (Sun et al. 2020). Melatonin is a secondary metabolite and is known to improve stress tolerance in plants by stimulating antioxidant activities (Marioni et al. 2008; Debnath et al. 2020). The quality, visual attractiveness and nutritional properties of *Punica granatum* L. sap have been found to be affected by the reduction of bioactive compounds such as anthocyanins and punicalagin under drought stress (Mena et al. 2013). Spraying leaves with selenium NPs increased phenolic content and improved the quality of drought-affected fruits of *P. granatum* (Zahedi et al. 2021).

6.4.2 NPs as Elicitors of Phytopharmaceuticals

Controlled elicitation is a strategy to increase the production of important secondary metabolites. As described in the previous sections, plants recognize different types of NMs and induce their secondary metabolism, which opens a new opportunity to improve the production of pharmaceutically important compounds in medicinal plants (Marslin et al. 2017; Shakya et al. 2019; Kruszka et al. 2020; Rivero-Montejo et al. 2021). Elicitation of several classes of secondary metabolites such as glucosinolates, terpenes and alkaloids have been reported to be obtained using NPs. The chemical structure of some pharmaceutically important secondary metabolites elicited using NMs is shown in Fig. 6.2.

6.4.2.1 Flavonoids

Flavonoids are natural bioactive compounds found predominantly in various parts of plants and have been attributed to various pharmacological and therapeutic properties (Panche et al. 2016). In *Momordica charantia* L., an increase in flavonoid concentration induced by 5 mg/L Ag NPs was observed (Chung et al. 2018c). Stimulation of *Thymus daenensis* Celak. plant cells with SWNT increased the total flavonoid content (Samadi et al. 2021). Quercetin is an important and abundant flavonoid from plants with rich pharmaceutical properties such as antitumor, anti-infective, anti-inflammatory and antioxidant activities (Qi et al. 2020). Increased quercetin content was observed in shoots and roots of *Nigella arvensis* L. treated with 50 mg/L NiO NPs (Modarresi et al. 2020). The level of several flavonoid aglycones like apigenin, kaempferol and quercetin was increased upon treatment with the Ag, Au, Cu and Pd NPs treatment, whereas flavonoid glucosides like quercetin 3-O-hexoside or quercetin 3-O-malonylhexoside was elicited by CuO NPs treatment in *H. perforatum* L. cell suspension cultures, (Kruszka et al. 2022). Anthocyanins are another subgroup of flavonoids and play an important role in the nutraceutical, pharmaceutical and food industries. After the application of ZnO NPs in the shooting culture of *Lilium ledebourii* (Baker) Boiss., an increase in anthocyanin concentration was observed, and the effect of polyphenol induction was dose-dependent (Chamani et al. 2015). Similarly, stimulation with SiO_2 NPs increased the concentration of the anticancer flavonoids xanthomicrol, isocaempferide, and cirsimaritin in the hairy roots of *Dracocephalum kotschyi* Boiss. (Nourozi et al. 2019b). Treatment of *D. kotschyi* cell suspension cultures with Fe_3O_4 magnetite NPs increased the content of rosmarinic acid, naringin, carvacrol, rutin, quercetin, apigenin and thymol (Taghizadeh et al. 2021).

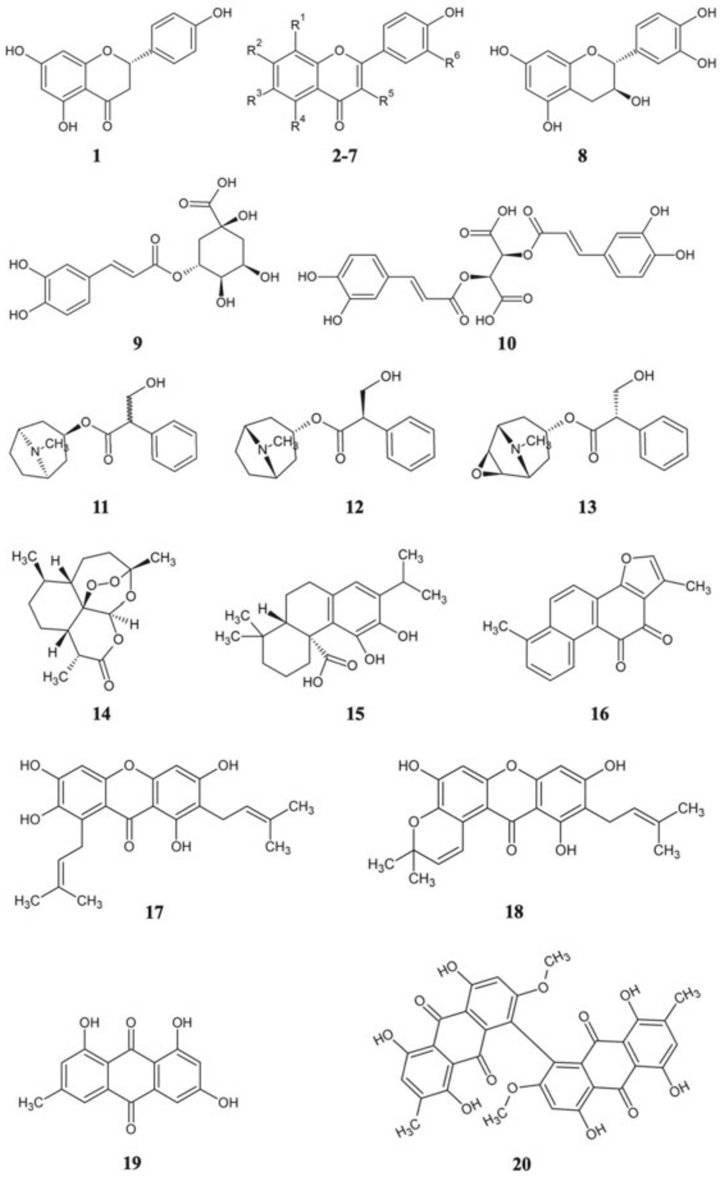

Fig. 6.2 Some of the pharmaceutically important secondary metabolites elicited from medicinal plants using NMs; (1) naringenin, (2) apigenin (R^1 = H, R^2 = OH, R^3 = H, R^4 = OH, R^5 = H, R^6 = H), (3) cirsimaritin (R^1 = H, R^2 = OMe, R^3 = OMe, R^4 = OH, R^5 = H, R^6 = H), (4) xanthomicrol (R^1 = OMe, R2 = OMe, R3 = OMe, R4 = OH, R5 = H, R6 = H), (5) kaempferol (R1 = H, R2 = OH, R^3 = H, R^4 = OH, R^5 = OH, R^6 = H), (6) isokaempferide (R^1 = H, R^2 = OH, R^3 = H, R^4 = OH, R^5 = OMe, R^6 = H), (7) quercetin (R^1 = H, R^2 = OH, R^3 = H, R^4 = OH, R^5 = OH, R^6 = OH), (8) catechin, (9) chlorogenic acid, (10) cichoric acid, (11) atropin, (12) hyoscyamine, (13) scopolamine, (14) artemisinin, (15) carnosic acid, (16) tanshinone, (17) γ-mangostin, (18) garcinone B, (19) emodin, (20) fusaroskyrin. (Figure constructed by D. Kruszka)

6.4.2.2 Phenolic Acids

Phenolic acids are an important group of plant secondary metabolites with a wide range of bioactivities, including anticancer, anti-inflammatory, neuroprotective, antioxidant, and antimicrobial activities (Kiokias et al. 2020). The phenolic acids, such as chlorogenic acid, coumaric acid, gallic acid, and tannic acid, were accumulated after the callus of *Prunella vulgaris* L. was exposed to Ag, Au, and Ag/Au NPs (Fazal et al. 2016). Moreover, Ag NPs induced the biosynthesis of phenolic acids more strongly than $AgNO_3$ in the hairy root culture of *Cucumis anguria* L. (Chung et al. 2018b). Ag and Cu NPs stimulated the secretion of hydroxycinnamic acid and hydroxybenzoic acid derivatives from *H. perforatum* cells into media of cell suspension cultures (Kruszka et al. 2022).

6.4.2.3 Glucosinolates

Glucosinolates are a group of Sulphur-containing hydrophilic secondary metabolites found primarily in members of the Brassicaceae and related families (Poveda et al. 2020; Wu et al. 2021). Glucosinolates exhibit some pharmacological bioactivities such as anti-inflammatory, antimicrobial, cholinesterase inhibitory, antioxidant and anticancer properties (Maina et al. 2020). Ag NPs induced biosynthesis of glucosinolates, a group of compounds responsible for response to pathogen attack, in addition to phenolic compounds in seedlings of *Brassica rapa* L. (Thiruvengadam et al. 2015). Treatment of hairy roots of Chinese cabbage with CuO NPs increased the accumulation of glucosinolates (Chung et al. 2018c). Moreover, the extracts of hairy roots released showed higher antimicrobial activity compared to the control.

6.4.2.4 Terpenoids

Terpenes and terpenoids are biogenic volatile organic compounds of plant secondary metabolites with high biological activity against various human diseases (Kim et al. 2020). The production of monoterpenes (linalool and linalyl acetate) in shoot cultures of *Mentha longifolia* L. grown under the influence of Co (0.8 mg/L) and Cu (0.5 mg/L) NPs (Talankova-Sereda et al. 2016). They reported that the higher production of essential oils corresponded with the growth index (Talankova-Sereda et al. 2016). Artemisinin, one of the important pharmaceutical compounds used as antimalarials, was induced by 2.5 and 5 mg/L Co NPs in *A. annua* cell culture (Ghasemi et al. 2015). Similar results were obtained after stimulation of *A. annua* hairy root culture by Ag-SiO_2 core–shell nanostructures (Zhang et al. 2013). A stimulatory effect of 8–21 nm Ag NPs on the increased production of diosgenin was observed in *Trigonella foenum-graecum* L. seedlings (Jasim et al. 2017). ZnO NPs (0.1–10 mg/L) increased the biosynthesis of rebaudioside-A and stevioside in shoot cultures of *Stevia rebaudiana* (Bert.), in addition to the induction of oxidative stress (Javed et al. 2017). Similarly, chitosan nanofibers and cellulose nanofibers increased the production of betulinic acid and betulin in cell suspension cultures of *Betula pendula* Roth (Vahide et al. 2021).

6.4.2.5 Alkaloids

Alkaloids are a large group of plant secondary metabolites with nitrogen atom(s) in their structure that exhibit a wide range of medicinally important bioactivities (Eguchi et al. 2019). Ag NPs induced the biosynthesis of atropine alkaloid in hair root culture of *Datura metel* L. and the highest level of atropine was detected after 48 h of treatment (Shakeran et al. 2015). This NP -based elicitor was better than $AgNO_3$ and two other biotic elicitors (*Staphylococcus aureus* F. J. Rosenbach and *Bacillus cereus* Frankland & Frankland). Hyoscyamine and scopolamine levels were significantly increased 24 h after application of 450–1800 mg/L Fe_2O_3 NPs in the hairy root culture of *Hyoscyamus reticulatus* L. (Moharrami et al. 2017). SiO_2 NPs triggered the production of tropane alkaloids (hyoscyamine and scopolamine) in hair root cultures of two *Hyoscyamus* species namely, *H. reticulatus* and *H. pusillus* L. (Hedayati et al. 2020). Cell suspension cultures of *Corylus avellane* produced more taxol and baccatin III after treatment with 5 mg/L Ag NPs (Jamshidi and Ghanati 2017). Available examples of the elicitation of pharmaceutically important secondary metabolites using NPs are summarized in Table 6.3.

6.4.2.6 Xanthones

Xanthones are bioactive secondary metabolites that possess antibacterial, antifungal activities, and could inhibit acetylcholinesterase, butyrylcholinesterase and tyrosinase (Badiali et al. 2018; Tusevski et al. 2018). Xanthones also possess neuroprotective activities (Xu et al. 2016; Velingkar et al. 2017). Ag, Au, Cu, Pd and CuO NPs stimulated accumulation of prenylated derivatives of xanthones (γ-mangostin, garcinone B and hyperxanthone C), whereas glycosylated xanthones (eg.: mangiferin, homomangiferin, neomangiferin) content was increased after Au, Cu and Pd NPs treatment in cell suspension system of *H. perforatum* L. (Kruszka et al. 2022).

6.4.2.7 Anthraquinones

Antidepressant activities of *H. perforatum* L. extracts are attributed to naphthodianthrones/ anthraquinones such as hypericin or pseudohypericin (Velingkar et al. 2017). Hypericin content was increased by TiO_2-perlite nanocomposite treatment in *H. perforatum* L. shoot cultures (Ebadollahi et al. 2019). Emodin and emodin anthrone contents were respectively increased by Pd and CeO_2 NPs treatment in *H. perforatum* L. cell suspension cultures (Kruszka et al. 2022). In the above study, a 98.6-fold increase of fusaroskyrin after Ag NP treatment was also reported.

Table 6.3 Nano-elicitation of pharmaceutically important plant secondary metabolites in medicinal plants

Compound	Application/uses	Plant species	NPs	Concentration (mg/L)	Reference
Artemisinin	Antimalarial	*Artemisia annua* L.	Ag-SiO_2	100–2000	Zhang et al. (2013))
			Co	0.25–5	Ghasemi et al. (2015)
Atropine	Antispasmodic, anti-Parkinson and cycloplegic drug	*Datura metel* L.	Ag	20	Shakeran et al. (2015)
Bacoside A	Nootropic	*Bacopa monnieri* L.	ZnO	1	Bhardwaj et al. (2018)
Carnosic acid	Antioxidative and antimicrobial	*Rosmarinus officinalis* L.	Ag	200	Hadi Soltanabad et al.(2020)
Catechin,	Iron-chelating, anti-oxidant, anti-inflammatory and anticancer	*Cucumis anguria* L.	Ag	0.5–2	Chung et al. (2018b)
Chlorogenic acid	Antioxidant, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, anti-microbial, anti-hypertension	*Dracocephalum moldavica* L.	TiO_2	30	Kamalizadeh et al. (2019)
Cichoric acid	Anti-cancer, anti-obesity, antiviral, and anti-diabetic	*Echinacea purpurea* L.	Ag	2	Ramezannezhad et al. (2019)

(continued)

Table 6.3 (continued)

Compound	Application/uses	Plant species	NPs	Concentration (mg/L)	Reference
		Ocimum basilicum L.	CuO	10	Nazir et al. (2021)
Cirsimaritin	Anticancer	*Dracocephalum kotschyi* Boiss	Fe	75	Nourozi et al. (2019a)
Diosgenin	Used in the treatment of cancers, hyperlipidemia, inflammation, and infections	*Trigonella foenum-graecum* L.	Ag	0.2	Jasim et al. (2017)
Glaucine	Antihyperlipidemic, antidiabetic, antioxidant, antiobesity, antitussive and antivirus	*Nigella arvensis* L.	NiO	1000	Modarresi et al. (2020)
Glucosinolate	Antioxidant, anti-inflammatory and antimicrobial	*Brassica rapa* L.	Ag	1 -10	Thiruvengadam et al. (2015)
			CuO	100	Chung et al. (2018a)
Glycyrrhizin	Anti-inflammatory	*Glycyrrhiza glabra* L.	CuO	0.79	Oloumi et al. (2015)
Gymnemic acid	Antidiabetic, antisweetener and anti-inflammatory	*Gymnema sylvestre* (R.Br)	CuO	3	Chung et al. (2019)
Hydroxybenzoic acid	Antimicrobial, antifungal, antisickling, and estrogenic	*Momordica charantia* L.	Ag	5	Chung et al. (2018c)
Hydroxycinnamic acid	Antioxidant, anti-collagenase, anti-inflammatory, antimicrobial and anti-tyrosinase	*Momordica charantia* L	Ag	5	Chung et al. (2018c)

(continued)

Table 6.3 (continued)

Compound	Application/uses	Plant species	NPs	Concentration (mg/L)	Reference
Hyoscyamine	Mydriatic, antispasmodic, anticholinergic, analgesic and sedative	*Hyoscyamus niger* L.	TiO_2	40	Ghorbanpour et al. (2015)
		Hyoscyamus reticulatus L.	Fe	450–900	Moharrami et al. (2017)
			ZnO	100	Asl et al. (2019)
Hypericin	Antidepressant, antineoplastic, antiviral and antimicrobial	*Hypericum Perforatum* L.	TiO_2/perlite	25–200	Ebadollahi et al. (2019)
Isokaempferide	Hepatoprotective, antimicrobial and antiproliferative	*Dracocephalum kotschyi Boiss*	Fe	75	Nourozi et al. (2019a)
Kaempferol	Antioxidant, anti-inflammatory and anticancer	*Amaranthus caudatus* L.	Ag	50	Azeez et al. (2017)
Khusimol	Antibacterial	*Vetiveria zizanioides* L.	TiO_2	90	Shabbir et al. (2019)
Lignan	Anti-inflammatory, antioxidant and antitumor	*Linum usitatissimum* L.	TiO_2	150	Karimzadeh et al. (2019)
			ZnO	100	Abbasi et al. (2019)
			Ag	0.03	Zahir et al. (2019)
Linalool	Anti-inflammatory, anticancer, antihyperlipidemic, antimicrobial, antinoceptive, analgesic, anxiolytic, antidepressant and neuroprotective	*Mentha longifolia* L.	Co, Cu	0.5- 0.8	Talankova-Sereda et al. (2016)

(continued)

Table 6.3 (continued)

Compound	Application/uses	Plant species	NPs	Concentration (mg/L)	Reference
Lycopene	Antioxidant	*Solanum lycopersicum* L.	Cu	250	López-Vargas et al. (2018)
Menthol	Analgesic, antiseptic, antibacterial and antifungal	*Mentha piperita* L.	Cu	1000	Lafmejani et al. (2018)
			TiO_2	150	Ahmad et al. (2018)
Myricetin,	Myricetin, iron-chelating, anti-oxidant, anti-inflammatory and anticancer	*Cucumis anguria* L.	Ag	0.5–2	Chung et al. (2018b)
Naringenin	Anti-dyslipidemic, anti-obesity, anti-diabetic and antifibrotic	*Cucumis anguria* L.	Ag	0.5–2	Chung et al. (2018b)
Quercetin	Anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, and psychostimulant	*Cucumis anguria* L.	Ag	0.5–2	Chung et al. (2018b)
		Amaranthus caudatus L.	Ag	50	Azeez et al. (2017)
		Salvia tebesana Bunge	TiO_2	60	Shoja et al. (2022)
Rosmarinic acid	Hepatoprotective, anti-inflammatory, neuroprotective and antioxidant	*Dracocephalum moldavica* L.	TiO_2	30	Kamalizadeh et al. (2019)
		Saponaria officinalis L.	TiO_2	100	Hedayati et al. (2022)
Saponin	Decrease blood lipids, lower cancer risks, and lower blood glucose response	*Calendula officinalis* L	Ag	43	Ghanati and Bakhtiarian (2014)

(continued)

Table 6.3 (continued)

Compound	Application/uses	Plant species	NPs	Concentration (mg/L)	Reference
Saponin	Anti-inflammatory,antibacterial, antifungal, antiviral,insecticidal, anticancer, cytotoxic and molluscicidal	*Bacopa monnieri* L.	Cu	30	Lala (2020)
Scopolamine	Mydriatic, antispasmodic, anticholinergic, analgesic and sedative	*Hyoscyamus niger* L.	TiO_2	40	Ghorbanpour et al. (2015)
		Hyoscyamus reticulatus L.	Fe	450–900	Moharrami et al. (2017)
			ZnO	100	Asl et al. (2019)
Silymarin	Used in the treatment of hepatic disorders, anti-carcinogenic	*Silybum marianum* L.	ZnO	0.15	Shehzad et al. (2021)
Steviol glycosides	Antioxidant	*Stevia rebaudiana* (Bert.)	CuO	20	Ahmad et al. (2020)
Steviol glycosides		*Stevia rebaudiana* (Bert.)	ZnO	1	Javed et al. (2017)
Stevioside	Anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory	*Stevia rebaudiana* (Bert.)	Ag	45	Golkar et al. (2019)
Syringic acid	Anti-oxidant, antimicrobial, anti-inflammatory and antiendotoxic	*Cucumis anguria* L.	Ag	0.5–2	Chung et al. (2018b)
Tanshinone	Antioxidant activity, anti-inflammatory activity, cardiovascular effects, and antitumor activity	*Salvia miltiorrhiza* Bunge	Ag	30	Ma et al. (2020)

(continued)

Table 6.3 (continued)

Compound	Application/uses	Plant species	NPs	Concentration (mg/L)	Reference
Taxanes	Antineoplastic	*Corylus avellana* L.	Ag	2–10	Jamshidi and Ghanati (2017)
Thymoquinone	Hepatoprotective, anti-inflammatory, antioxidant, cytotoxic and anti-cancer chemical	*Nigella sativa* L.	TiO_2	100	Kahila et al. (2018)
Tryptanthrin	Anti-inflammatory, antibacterial and anticancer	*Isatis Constricta* P.H.Davis	Ag	2	Karakaş (2020)
Vanillic acid	Anti-inflammatory, antihypertensive and antioxidant	*Cucumis anguria* L.	Ag	0.5–2	Chung et al. (2018b)
Withanolide	Anti-inflammatory, anticancer and neuroprotective, antidepressant	*Withania somnifera* (L.) Dunal	Zn-Ag (19:1)	20	Singh et al. (2019)
Xanthomicrol	Anticancer	*Dracocephalum kotschyi* Boiss	Fe	75	Nourozi et al. (2019a)
Xanthones	Neuroprotective, anti-inflammatory, antibacterial, antioxidant	*Hypericum perforatum* L.	Ag, Au, Cu, Pd	25	Kruszka et al. (2022)

6.5 Conclusion and Prospects

Plant secondary metabolites play an important role in plant's fitness and adaptation. Therefore, alteration of secondary metabolism by NPs could affect crop quality and agricultural productivity. The pharmacological properties of several medicinal plants are attributed to the crude extracts or decoctions and not to the individual compounds. Therefore, any alteration in the secondary metabolism of medicinal plants would affect their pharmacological potential and market value. Among the numerous compounds accumulated in plants, many of them possess antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective, antidepressant, antioxidant, neuroprotective and anticancer properties. A better understanding of the effects of NPs on plant secondary metabolism would allow us to develop strategies to help plants cope with the increasing presence of NPs in the environment and to develop new molecular pharmaceutical tools (Fig. 6.3).

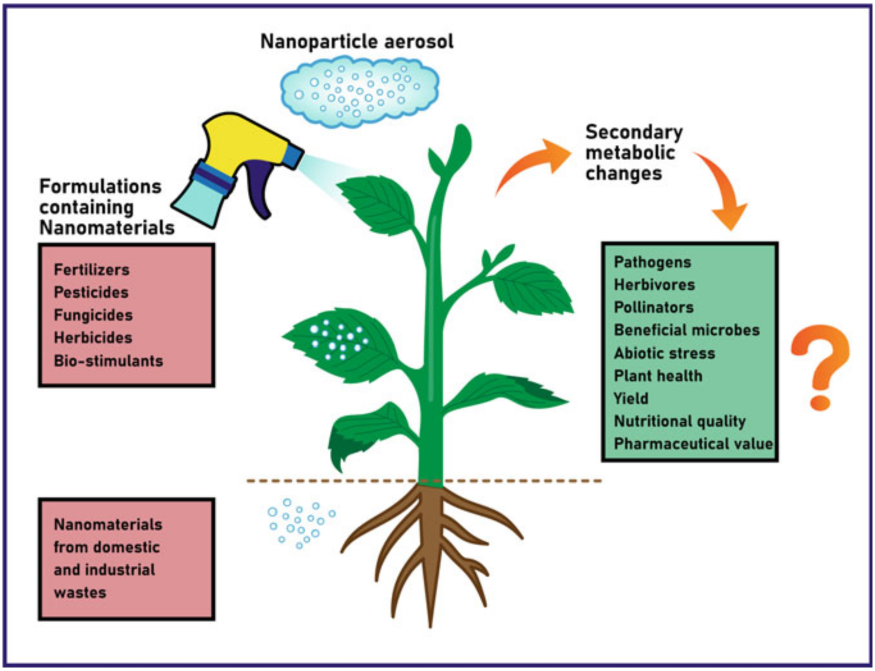

Fig. 6.3 The potential effects of secondary metabolic changes caused by NMs on other associated plant parameters. NMs can enter plants in both intentional and unintentional ways. Although changes in secondary metabolism could affect plants' ability to protect themselves against pathogens, herbivores, and adverse environmental conditions, as well as their ability to communicate with beneficial microbes, more research is needed to understand the exact consequences (Figure constructed by G. Franklin)

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Chapter 7
Toxic Effects of Nanomaterials on Plant Cellular Mechanisms

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Abstract Several nanomaterials (NMs) with great potential have been introduced to revolutionize the agricultural industry and are characterized by several advantages and disadvantages. The toxic mechanisms of NMs to the plants depend on their physicochemical characteristics, such as nature, morphology, crystallinity, the surface area of the types involved, concentration, being a definitive property to make NMs toxic or non-toxic. General mechanisms of NMs plant toxicity to the plant cells are growth inhibition, seed germination, root elongation, biomass reduction, leaf numbers alteration, and fresh plant weight changes. NMs exposure to plants results in various side effects, including reactive oxygen species (ROS) generation, lipid

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peroxidation, disruption of redox homeostasis, DNA, and membrane damage. Therefore, the NMs can induce oxidative stress, interfere with plant growth, and induce genotoxicity. Despite numerous benefits of NMs for agriculture, exploring the risks of nanotoxicity due to the incorporation of NMs into food for human consumption has become imperative as engineered and incidental nanomaterials can cause future health problems. This chapter presents the results and progress made towards the toxicity of diverse nanomaterials to plant cellular mechanisms and future research directions to explain the roles of the NMs properties to ensure safe use in the agriculture sector, being this sector one of the main foods sources for human.

Keywords Abiotic stress · Cellular mechanisms · Nanomaterials · Nanomaterial's uptake routes · Nanotoxicology · Plants · Toxicology

7.1 Introduction

The relationship between plants and nanomaterials (NMs) could be interpreted as an accidental relationship due to their exposure to diverse compounds and materials in the nanoscale. Nevertheless, the use of the word "nano" goes beyond the size. It relates the morphology, crystallinity, reactivity, surface area, concentration, and all the chemical and physical characteristics that made the NMs unique compared to their bulk counterparts (Dev et al. 2018).

Nanomaterials represent greatly the Nanotechnology and are helpful to construct new materials with different sizes 1–100 nm at least in one spatial dimension (Mageswari et al. 2016; Murr 2017). The applications of these materials have been in all possible areas throughout the past decade in medicine, pharmaceutics, cosmetology, environment remediation, electronics, aerospace sciences, automotive industry, materials engineering, textiles, food sciences, biotechnology, and agriculture. In the agri-food sector, NMs have been utilized as nano-fertilizers, nano-pesticides, nano-additives, and nano-elicitors. All of them improve yield and generate better functional food efficiently and rapidly (Mathur and Roy 2020).

Although the use of NMs has increased, their toxicity remains unknown in many cases. Despite their known toxic potential on fish, algae, bacteria, vertebrates, and invertebrates, much research is still lacking in this area. Jamil et al. (2018). Also, the use of NMs in the agri-food sector gives the uncertainty and the possibility of toxicity due to the materials uptake by the plants' fruits and eventually reach the human being (see Fig. 7.1).

7.1.1 NMs Classification

As mentioned earlier, the uptake of the NMs in plants will also depend on their chemistry classification NMs of mineral origin can be classified into two groups

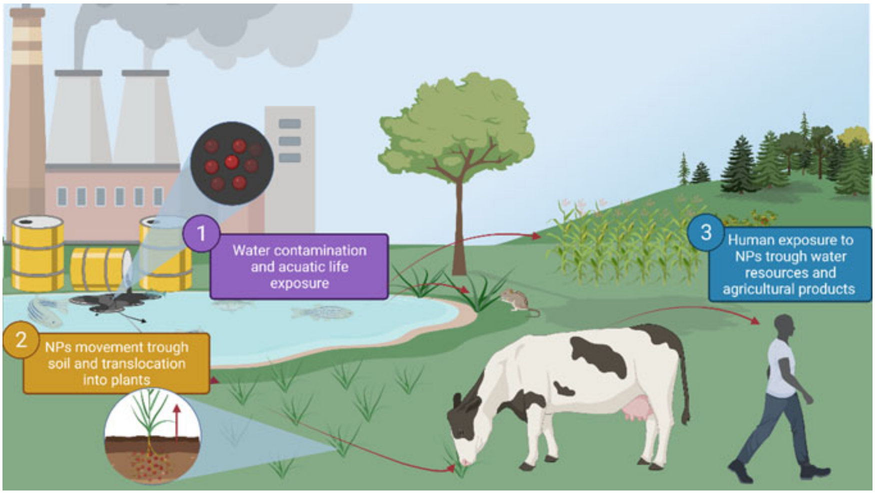

Fig. 7.1 NMs uptake pathway along the food chain (Created by K. Esquivel with BioRender)

according to their chemical composition: inorganic NMs do not contain carbon, and organic NMs are those that contain hydrocarbons. Inorganic NMs are based on metal oxides/hydroxides, metals, and transition metal chalcogenides (TMC-NM). Organic NMs include compact polymers such as nanospheres, nanocapsules, micelles, liposomes, dendrimers, hybrid NMs, and carbon-based nanomaterials such as fullerenes, graphene, and carbon nanotubes. Moreover, carbon-based nanomaterials are considered a separate class of nanomaterials (Kumar et al. 2019), as shown in Fig. 7.2. Furthermore, NMs can be classified according to the number of their dimensions. 0D includes materials with dimensions at a nanoscale level such as nanoparticles (NPs), 1D are NMs having at least one dimension at macroscale levels such as nanofibers and nanowires, 2D are materials with two dimensions at macroscale level such as nanosheets and thin films. Finally, 3D are bulk materials. Kumar and Sinha Ray (2018), Paramo et al. (2020).

7.1.1.1 Metal-Based Nanomaterials (Metallic and Oxides NMs)

Usually, the plants are not as a few years ago encountered by metallic or metallic oxides NMs during their evolution, so there are no defensive mechanisms against these NMs. Therefore, the toxicity of metallic and metallic oxides NMs has emerged as a worldwide concern (Jamil et al. 2018). Some of the most toxicity studied NMs for agriculture applications and classified as metal-based are gold (Au), silver (Ag), iron (Fe), zinc (Zn), copper (Cu), magnesium (Mn), nickel (Ni), silicon (Si); and for the metallic oxide, NMs are TiO_2, SiO_2, ZnO, Fe_3O_4, CeO_2, CuO, Al_2O_3, FeO (Al-Amri et al. 2020; Jahan et al. 2018; Mathur and Roy 2020; Picó et al. 2017).

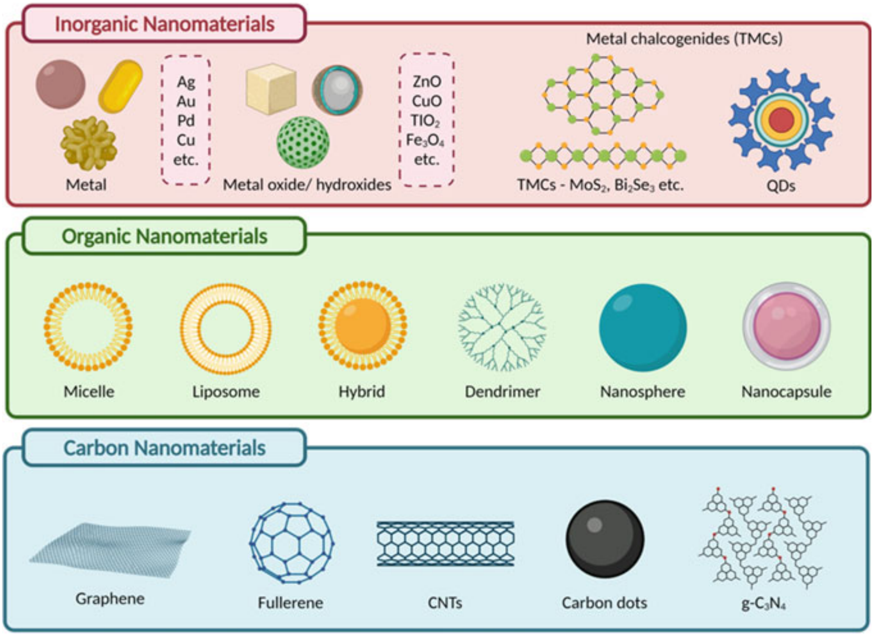

Fig. 7.2 Nanomaterial's classification (Created by K. Esquivel with BioRender)

As mentioned, NMs are recommended as an additive due to their efficiency in their use as a nutrient, for plant growth, in seed germination, and their use for soil remediation, mainly for nutrient-deficient soils. However, there is still concern about the transfer of NMs to edible parts of the plant such as leaves, seeds, and fruits; since it is the one that can have access to animals and humans through the food chain, further research is required before its application on a larger scale (Al-Amri et al. 2020). Despite the rapid progress in the study of plant interaction with NMs, the determination of phytotoxicity of metallic NMs is complex due to the potential dissolution and uptake of metallic ions from metal oxide NMs. The determination of relevant components of the plant to NMs uptake is challenging due to the different mobility of the NMs. Moreover, the ability of specific plants to absorb NMs is currently unpredictable and unknown (Ullah et al. 2020).

7.1.1.2 Carbon-Based Nanomaterials (MWCNT and SWCNT)

Carbon-based NMs are widely used as conductive materials that mediate biological contamination removal, optical devices, medicine production, and drug delivery. In this type of NMs, it is possible to find fullerenes C_{70}, fullerols ($C_{60}(OH)_{20}$), graphene, nanohorns, single wall and multi-wall carbon nanotubes (SWCNTs, MWCNTs) (Dev et al. 2018; Mageswari et al. 2016; Murr 2017; Samadi et al. 2021). In the agriculture sector, some investigations demonstrated that carbon-based NMs minimized the oxidative stress in plants related to the morphology, solubility, functionality, concentration, and type of plant applied to (Samadi et al. 2021).

Overall, in some cases, carbon-based NMs increase plant health by decreasing the hydrogen peroxide (H_2O_2) level and protein and lipid oxidation. However, no comprehensive review deals with the carbon-based NMs properties and their role in physiological and biochemical processes and pharmacological active compound accumulation in plants (Samadi et al. 2021).

7.2 Uptake Routes of NMs in Plants

The difference in the adsorption behavior of NMs and bulk materials lies, apart from the size, with the chemical interactions between the charged ions of the bulk materials and the soil since these charged ions form stronger electrostatic interactions than uncharged NMs. On the other hand, the size of the NMs also plays a vital role in the time it will remain in the soil, as NMs will have longer residence times, unlike bulk materials, which decreases the leaching of nutrients to the soil, increasing their availability for plant uptake (López-Moreno et al. 2018). The application of the NMs over the plants can be made by irrigation, leaves spray, and direct injection when the plants are in the soil. For the hydroponic treatments, the NMs are suspended in the nutritional solution, and those uptakes along the plants' roots can be translocated along the stem, leaves, and even fruits (Paramo et al. 2020). So far, the most accepted model by which NMs act with plants is cell penetration. This has been seen at the in vitro level. However, the exact mechanism of uptake has not been understood. It has been reported that the endocytosis pathway plays an essential role for the uptake of NMs (Núñez and de la Rosa-Álvarez 2018).

In vivo studies where NMs have been incorporated have been associated with moisture and nutrient uptake and depend on the plant development stage. In the embryonic root, the absorption of NMs occurs, and these are incorporated into the plant. In the aerial parts of the plant, the adsorption of NMs occurs, according to reports, by two main routes, the first by cuticular pathways and the second by stomatal pathways, diffusion of lipophilic chemicals and transport of polar or ionic solutes (Núñez and de la Rosa-Álvarez 2018). Nevertheless, all the information gained in the past decades about this topic remains with the same observations, the final mechanisms of toxicity and plant uptake of the NMs will depend on the type, physics, and chemical NMs characteristics along with the type of plant and type of NMs application over the proper plant or crop (Gwinn 2019).

The anatomical structure of the plant surface favors the deposit of different NMs. In shoots with epicuticular structures, the lotus effect occurs, which consists in the decrease of wettability, and this allows the deposit of different NMs. Due to the different openings of the different structures that make up the plant, NMs could enter some structures: cuticle, mucilage, exudates, cell wall, cell membrane, and proteins

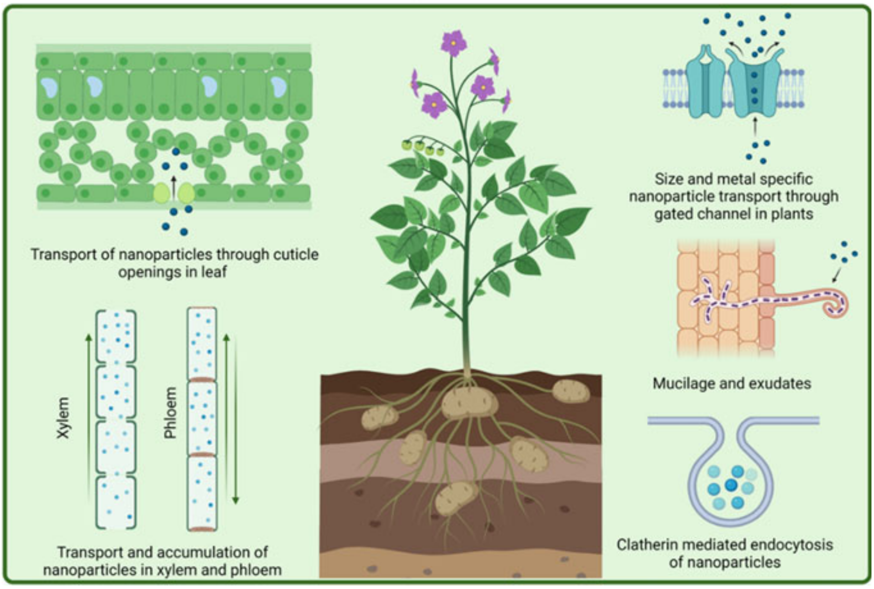

Fig. 7.3 Uptake routes of the NMs in plants (Created by K. Esquivel with BioRender)

(transporters, aquaporins, and ion channels). The latter allows NMs to enter the cell (Fig. 7.3).

Uptake of metal NPs occur through specific ion transporters (Dev et al. 2018). NMs attach to specific cell membrane receptors and invaginate through a group of vesicle-forming proteins called clathrin or adaptin. This type of mechanism occurs in tiny NPs as a clathrin-coated vesicle has a size in the range of 70–120 n (Dev et al. 2018).

7.3 Nanomaterials and Their Toxic Effect on Plants

Nanotechnology has been used in different fields; however, knowledge on its mechanism and toxic effects has not yet been well understood. The toxicity of nanomaterial, mainly in plants, is affected by different factors such as size, solubility, shape, surface charge, concentration, composition, and thermodynamic properties. Although in recent years, the interest in the application of nanomaterials in agriculture has been mainly devoted to improving yields and germination of seeds, crop production, detection of pesticide residues, and disease control.

However, the negative impact and toxicity in different plant species have not been discussed in depth (Anjum et al. 2019; Elnashaie et al. 2018; Jeevanandam et al. 2018; Lin and Xing 2007; Roco 2011).

One of the recent multidisciplinary fields is nano ecotoxicology, dedicated to natural and synthetic materials with sizes on the nanoscale and their potentially dangerous effects on the environment and health in living systems (Cattaneo et al. 2009; Boros and Ostafe 2020). Synthetic nanomaterials have been intentionally generated for specific applications which are considered potentially toxic since there has not been proper control of these; In addition, only some countries have been concerned with establishing some standards for the application of nanomaterials, such as the United States, Canada, and China. However, a thorough and deep study must be conducted since the most used particles in the case of pesticide and nutrient control are Ag, ZnO, Fe_2O_3, TiO_2, NiO, Co, and Si, which are not adequately regulated (Sardoiwala et al. 2018).

To avoid the unnecessary usage of plants, in vitro techniques have been used mainly for medicinal purposes, food additives, cosmetics since plants contain active ingredients facilitating rapid production and high quality in the plant, being able to extract metabolites without exploiting populations of wild plants (Verma et al. 2016). In vitro growth has excellent functionality for the evaluation of different parameters regarding the effects of nanoparticles such as the content of the nanoparticle, reactive oxygen species (ROS), cell death, cytotoxicity, enzymatic activity, therefore, to detect the effect of nanomaterials in different parts of the plant; however, the study of the potential impact of these nanomaterials on specific ecological processes has not yet been expanded.

Through in vitro culture technique, distinct nanomaterials have been used, such as metal oxide NPs; principally ZnO and TiO_2, carbon nanotubes (MWCNT), semiconductors, and polymeric nanoparticles (Hu et al. 2009; Panda et al. 2011; Bagherzadeh and Ehsanpour 2016; Verma et al. 2019; García-Sánchez et al. 2021) where metallic NPs have more significant toxicity on the plants since they trigger the formation of ROS. However, the use of nanoparticles has been evaluated as having lower cytotoxicity than ionic or colloidal species.

Although the importance in the ecological impact has not been observed in a response of a specific genetic relationship to nanomaterials, changes have been seen in the plant transcriptome (Van Aken 2015), due to metallic NPs such as ZnO, affecting genes related to oxidative stress as well as the inhibition of genes for the development and growth of roots and the response to pathogens (García-Sánchez et al. 2021; Pagano et al. 2016; Remédios et al. 2012) being of great importance the transmission of NPs from the first to the second generation through the seeds.

The application of nanotechnology in the agricultural sector has been proposed through an improvement in biological activity, where it has been used mostly in the seed, to improve its germination; however, inactivity times of certain species have already been perceived (Das et al. 2019; do Espirito Santo Pereira 2021; Lin and Xing 2007).

It is known that the toxicity of the nanoparticle is due to its size since the surface area is more significant concerning the unit of mass-radius, which allows the nanoparticle to react with its surroundings. Furthermore, the nanometric sizes of these particles can facilitate the entrance into the cell and have high chemical and biological reactivity. The rapid advancement in nanotechnology and the plants that are part of ecosystems has led us to study the interaction of NMs with seeds, plants, and crop soil. As several authors have described, whether a nanoparticle becomes toxic or creates oxidative stress has to do with its shape, size, and amount present (Al-Amri et al. 2020). Etesami et al. (2021) proposed that the safe amount used in agriculture research must be environmentally safe.

7.3.1 *Toxicity of Metal and Metal Oxide NMs*

Metallic NMs used in plant growth and development studies are of two types (1) metal oxides, which can influence plant growth and development; showing opposing effects, both beneficial and detrimental on the shoot and root growth, seed germination, biomass production, physiological and biochemical activities and (2) pure metals (Kumar et al. 2019). Plants interact with NPs through roots, and the uptake and translocation capacity of metal oxide NMs change concerning their concentration, bioavailability, solubility, and exposure time. The exact mechanism that causes plant defense against toxicity induced using metal oxides has not yet been addressed (Kumar et al. 2019).

NMs of metal compounds and metal oxides alter plants' morpho-anatomical, genetic, and physiological material when exposed to high concentrations, between 100 to 1000 mg/L, as they cause high ROS production. On the other hand, there are plant species that do not show physiological changes in the presence of this type of NMs. In contrast, cowpeas (*Vigna unguiculata* L.) show significant variations in the activity of antioxidant enzymes and an increase in heat shock regulatory proteins (Verma et al. 2018). Ag NPs can induce DNA damage in plants, causing the formation of chromatin bridges, stickiness, disturbed metaphase, and multiple chromosomal breaks (Debnath et al. 2018). In mustard (*Brassica nigra* L.), Ag NPs reduced seed germination rate, lipase activity, and sugar content in germination seeds. Ag NPs at 800 mg/L suppressed HO-1 (*Heme oxygenase*) expression, contrary at lower concentration (200–400 mg/L) a protective effect was observed, since increased transcription of hemeoxygenase-1 gene was scored, and it showed overproduction of HO-1. This resulted in increased mustard resistance to Hg toxicity (Maroufpoor et al. 2019). Recently, it has been identified that HO-1 plays an essential part in several physiological processes: dormancy break, seed germination, growth and developmental regulation, stomatal closure, and adaptation response to environmental stress (Amooaghaie et al. 2015).

Differential response to Ag NPs depends on plant species since, in the case of *Triticum aestivum* L. (wheat), a low amount of Ag (1–10 mg/L) decreased the fresh biomass production due to the root and shoot length reduction. The highest concentration, 10 mg/L, changed the expression of several genes mainly involved in cell defense mechanisms such as HCF136 protein and cytochrome b5 (Vannini et al. 2014). The exposure to 10 mg/L Ag NPs negatively affected the seedling growth and induced morphological modifications in root tip cells. Transmission electron microscopy (TEM) analysis suggests that the studied effects were due primarily to the release of Ag ions from Ag NPs. 2D-DE (two-dimensional DNA electrophoresis) profiling of roots and shoots treated revealed an altered expression of several proteins mainly involved in primary metabolism and cell defense (Maroufpoor et al. 2019; Vannini et al. 2014).

Regarding crucial agroeconomic cultivation, tomato (*Lycopersicon esculentum* L.) was treated with different concentrations of Ag NPs: 50, 100, 1000, 2500, 5000 mg/L, clearly decreasing the development of the roots, the activity of superoxide dismutase was higher, and the amount of chlorophyll was reduced (Kapinder et al. 2021). In addition, stressors on plants can affect their antioxidant activities, such as heavy metals so NMs may affect these physiological measures in similar or different ways (Song et al. 2013). The tomato treatments of 100 and 1000 mg/L Ag NPs solutions bore no fruit, although several flowers bloomed (Song et al. 2013). On the other hand, in *Spinacia oleracea* L., the treatment with TiO_2 stimulated photochemical reaction, and subsequent studies proposed a mechanism based on the penetration of the TiO_2 into the chloroplast. Followed by binding to the photosystem II and the beginning of the primary reaction, the charge separation, the amount of the chlorophylls a, chlorophylls b, and carotenoid molecules were reduced by higher concentrations of Fe_3O_4 (10–50 μL/L) in *Zea mays* L. In contrast, the chlorophylls a/b ratio and the photosynthesis process were decreased by low or high concentrations of Fe_3O_4 (Chichiriccò and Poma 2015). More effects related to metal-based NMs are presented in Table 7.1.

Most of the studies on the phytotoxicity of metal NPs have been carried out using Ag NPs. However, more studies are needed to understand how the migration of NPs occurs and all the changes that can occur at the cellular level. There are still few studies to try to understand the mechanism of NPs in the regulation of metabolites.

7.3.2 Toxicity of Carbon-Based NMs

Investigations have shown that treatments with multi-walled carbon nanotubes (MWCNTs) in leaf cell cultures of *Arabidopsis thaliana* L. and *Oryza sativa* L. decrease superoxide dismutase (SOD) activity, which is associated with a decrease in chlorophyll synthesis and at the same time an increase in the production of reactive oxygen species (ROS) and an increase in the apoptotic process. On the other hand, single-walled carbon nanotubes (SWCNTs) are known to penetrate the membrane of spinach chloroplasts, increasing electron flow and their photosynthetic activity (Jahan et al. 2018). Plant-enclosed nanotubes have been proposed to be used as nitric oxides (NOx) detectors because SWCNTs were shown to be sensitive to NOx. When *Allium cepa* L. was exposed to fullerol (C_{60} $(OH)_{24}$), it resulted in cell necrosis and generation of ROS, leading to the loss of membrane integrity (Chichiriccò and Poma 2015; Kumar et al. 2019). More examples are listed in Table 7.2. Phytotoxicity by nanoparticles of carbon compounds occurs mainly by blocking cell pores, leading to cellular changes by the migration of nanoparticles into the cell.

Table 7.1 Effect of metal-based NMs over diverse plant species

Nanomaterials	Effect	Plant	References
Ag NPs	Regulate the seed germination rate, enhance plant growth and diosgenin synthesis, increase root nodulation, and improve shoot parameters Biomass reduction of root and shoot affects leaf area, photosynthetic pigments' contents, chlorophyll fluorescence and soluble protein, shorter plant height, and lower grain weight Cause oxidative stress, ROS increase, reduce the size of the vacuole, which eventually reduces the cell turgidity and size Diminution the rate of transpiration Causes various types of chromosomal aberrations in both mitotic and meiotic cells Decreased sugar contents and caused significant foliar proline accumulation Caused metabolic disorders, inhibited the growth of the protonema, changed the thylakoid content Inhibited activities of glutathione reductase and dehydroascorbate reductase, higher ion leakage Decreases in the contents of micronutrients (Ca, Mg, B, Mn, Fe, Cu, and Zn); arginine and histidine Increased the malondialdehyde, H_2O_2, and total cytokinins in the leave´s contents Inhibit root gravitropism, reduce auxin accumulation in root tips, downregulated expression of auxin receptor-related genes Upregulation of genes involved in the ethylene signaling pathway Retarded the process of modulation, caused early senescence of root nodules Significant increases in the enzymatic activities accumulated more amino acids response to fungal infection Repressed transcriptional responses to microbial pathogens, resulting in increased bacterial colonization Toxicity increased with decreasing Ag NPs size	*Glycine max* L *Oryza sativa* L *Zea mays* L *Brassica oleracea var. capitata* L *Curcubita pepo* L *Arabdiopsis thaliana* L *Brassica nigra* L *Lupinus termis* L *Triticum aestivum* L *Physcomitrella patens* L *Lemna minor* L *Capsicum annuum* L *Allium cepa* L *Trigonella foenum-graecum L* *Cucumis sativus* L *Vicia faba* L *Solanum tuberosum* L *Vigna sinensis* L *Brassica juncea* L *Wolffia globose* Roxb *Spirodela polyrhiza* L *Solanum lycopersicum* L *Phaseolus radiatus* L *Asparagus officinalis* L *Vigna Radiata* L *Brassica Campestris* L	Li et al. (2017) Mazumdar and Ahmed (2011), Mirzajani et al. (2013), Mirzajani et al. (2014), Nair and Chung (2014) Pokhrel and Dubey (2013) Pokhrel and Dubey (2013) Hawthorne et al. (2012) García-Sánchez et al. (2015), Geisler-Lee et al. (2014), Kaveh et al. (2013), Kohan-Baghkheirati and Geisler-Lee (2015), Sun et al. (2017), Syu et al. (2014), Wang et al. (2013a, b), Wen et al. (2016) Amooaghaie et al. (2015), Vishwakarma et al. (2017) Al-Huqail et al. (2018) Abdelsalam et al. (2018), Dimkpa et al. (2013), Pallavi et al. (2016), Yang et al. (2018) Liang et al. (2018) Gubbins et al. (2011), Pereira et al. (2018) Vinković et al. (2017) Cvjetko et al. (2017), Kumari et al. (2009), Panda et al. (2011), Saha and Dutta Gupta (2017) Jasim et al. (2017) Tripathi et al. (2017a, b, c) Abd-Alla et al. 2016; Patlolla et al. (2012) Bagherzadeh Homaee and Ehsanpour (2016) Pallavi et al. (2016) Pallavi et al. (2016), Sharma et al. (2012) Zou et al. (2016) Jiang et al. (2014), Song et al. (2013) Lee et al. (2012), An et al. (2008) Mazumdar (2014) Mazumdar (2014)

(continued)

Table 7.1 (continued)

Nanomaterials	Effect	Plant	References
Au NPs	Root accumulation	*Raphanus sativus* L *Cucurbita pepo* L *Lolium perenne* L	Zhu et al. (2012)
Cu NPs	An increase in shoot biomass, concentrations above 100 mg/L, negatively affected chlorophyll content, biomass, and starch concentration Increased catalase, peroxidase, and polyphenol oxidase activity	*Capsicum annuum* L	Tabatabaee et al. (2021)
Mn NPs	Increase of nitrogen uptake, improve the growing seedling, root elongation	*Vigna radiata* L *Lactuca sativa* L	Pradhan et al. (2014) Liu et al. (2016)
Ni NPs	Higher activity of enzymes catalase and contents of glutathione and lipid peroxidation	*Solanum lycopersicum* L	Faisal et al. (2013)

Table 7.2 Effect of carbon-based NMs over diverse plant species

Nanomaterials	Effect	Plant	References
Single Wall Carbon Nanotubes (SWCNTs)	Elongation in the root, delayed flowering and yield, induced generation of ROS causing apoptosis	*Solanum lycopersicum* L *Nicotiana tabacum* L *Cucumis sativus* L *Lactuca sativa* L *Oryza sativa* L *Brassica oleracea* L *Daucus carota* L *Arabidopsis thaliana* *Allium cepa* L	Cañas et al. (2008) Khodakovskaya et al. (2012) Cañas et al. (2008) Cañas et al. (2008) Lin et al. (2009), Shen et al. (2010) Cañas et al. (2008) Cañas et al. (2008) Shen et al. (2010) Cañas et al. (2008)
Multi-Wall Carbon Nanotubes (MWCNTs)	Reduction in biomass delayed flowering Stimulates reactive oxygen species (ROS) generation affecting the cell proliferation, increased level of antioxidants and finally apoptosis in the cell, also causes chromatin condensation and shrinkage of cells	*Raphanus sativus* L *Lolium perenne* L *Zea mays L* *Onobrychis arenaria* DC *Curcubita pepo* L *Oryza sativa* L *Lactuca sativa* L *Brassica napus* L *Cucumis sativus* L	Lin and Xing (2007) Lin and Xing 2007 Lin and Xing (2007) Smirnova et al. (2011) Stampoulis et al. (2009) Tan et al. (2009) Lin and Xing (2007) Lin and Xing (2007) Lin and Xing (2007)
Fullerol [$C_{60}(OH)_{20}$]	Reduce root length	*Momordica charantia* L *Allium cepa* L	Chen et al. (2010)
$C_{70}((C(COOH))_2$	Root length reduced, promotes negative geotropism in roots	*Arabidopsis thaliana* L	Liu et al. (2010)
Fullerene nC_{60}	Decrease in normalized relative transpiration Blockage of root pores Structural change in the inner walls of endothelial cells Low concentrations decreased lindane uptake, and high concentrations promoted lindane adsorption	*Triticum aestivum* L	He et al. (2021)

(continued)

Table 7.2 (continued)

Nanomaterials	Effect	Plant	References
Nanodiamonds	Higher concentrations of NDs inhibited growth, generated malformation and exacerbated oxidative stress Improved photosynthesis was seen with increased chlorophyll production	*Chlorella pyrenoidosa*	Zhang et al. (2021)

7.4 Altered Enzyme Activity

It is well known that plants have two pathways to carry out antioxidant defense mechanisms: (1) the enzymatic and (2) the non-enzymatic. The first one includes the enzymes catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and dehydroascorbate reductase (DHAR). The non-enzymatic mechanism is the glutathione, ascorbate, thiols, and phenolic compounds (Kumar et al. 2019; Rajput et al. 2021). Interestingly, metallic oxide-NMs are reported to activate the antioxidant machinery in plants recorded enhanced activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) in wheat plants that are treated with Fe_3O_4. The TiO_2 induced catalase and glutathione reductase (GR) activities in water thyme (*Hydrilla verticillata* L.).

Enzymes are natural biocatalysts of a protein origin. The action of several enzymes carries out all biochemical reactions of cellular metabolism. Some studies have demonstrated that the presence of nanoparticles adversely affects the enzymes in plants. For example, it was demonstrated an apparent reduction in the activities of dehydrogenase and oxide-reductase in plants after the exposition to TiO_2, Al_2O_3 (Kapinder et al. 2021). Nevertheless, treatment with La_2O_3 or CuO has been demonstrated in zucchini (*Cucurbita pepo L.*) and tomato (*Solanum lycopersicum L.*) to inhibit root and shoot elongation and biomass, as well as induce ROS conducting to programmed cell death (Cota-Ruiz et al. 2020).

Nowadays, the NMs are considered a great source of stress factors for plants since there is a possibility to change the structure and the constitution of the cell wall and membrane of the plants (Juárez-Maldonado et al. 2021). Several studies have shown that the carbon and metallic NMs can produce stress in the plant, generating an excess of ROS. These ROS can affect proteins, lipids, carbohydrates, DNA and is well known to induce oxidative damage and alteration in ion transport across the cell membrane (Dev et al. 2018). These NMs can access the plant cells throughout the root system, composed of the xylem, phloem, and cortex (Sardoiwala et al. 2018). Considering the size of the NM, they can easily penetrate across the restriction barriers of plant surfaces and develop their cytotoxic effects (Sardoiwala et al. 2018). In some specific cases, the size of the NMs is too large to get access by the root system. Then these NMs use natural plant openings like flower stigma, hydathodes, and stomata (Dev et al. 2018).

Several NMs like TiO_2, Fe_3O_4, ZnO, $Al_2O_{3,}$ and CrO_3 and carbon-based NMs like carbon nanotubes have been tested for their effect produced in plants. Both *Arabidopsis thaliana* L. and wheat (*Triticum aestivum* L.) affect cell morphology, mitochondrial function, apoptosis process, and plasma membrane permeability. In general, nanoparticles changed the cell morphology and damaged membrane (Liu et al. 2021; Majeed et al. 2020; Sardoiwala et al. 2018).

Once the NMs are inside de cell, the metal-base of the NMs is converted into reactive metal ions, which react with functional groups present in proteins altering their biochemical activity (Dev et al. 2018). The large surface area of these NMs absorbs the different molecules such as nutrients and ions found in the milieu, resulting in indirect toxicity symptoms like chlorosis, wilting, abortion of fruit, sterile flowers, and drying of fruit (Jahan et al. 2018). Sometimes the production of ROS increases. This produces oxidative damage resulting in lipid peroxidation, DNA damage, and cell death. Another essential change due to the presence of NMs is the modification in the number of hormones such as cytokinin and auxins. On the other hand, it has been observed that CuO (10, 50, and 300 mg/L) produced adverse effects on a cotton field, causing significant decreasing changes in the concentrations of indole-3-acetic acid (IAA) and abscisic acid (ABA) (Hao et al. 2016; Jordan et al. 2018).

The jasmonic acid (JA) is an indicator of plant stress and can lead to the expression of several stress-related genes such as Dgl, LeAos3 (Hatami et al. 2019; Jordan et al. 2018). These changes upregulated the genes responsible for increasing the shikimic acid pathway, related to the production of secondary metabolites like alkaloids, aromatic metabolites, tannins, flavonoids, and lignin (Jordan et al. 2018). Recently, in rice (*Oryza sativa* L.), a reduction in the synthesis of phytohormones like abscisic acid (ABA), brassinolide (BR), and JA due to treatment with MWNTs (Multi-walled nanotubes), Fe-CNTs, and FeCO-CNTs (Jordan et al. 2018). In *Zea mays* L., metallic NMs block the transpiration and decline in hydraulic conductivities; in *Cucurbita, pepo* L. also reduces the transpiration. In green peas at a concentration from 0–500 mg/Kg of Zn nanoparticles (NPs) reduce the amount of chlorophyll until 85%, revealing that 200 mg/Kg causes toxicity effects. The first symptom was the absence of chlorophyll (leaves were changing from green to yellow), then loss of turgidity, and finally abscission of the leaves (Mukherjee et al. 2014).

7.5 Altered Gene Expression

The genotoxicity of NMs, referred to as any interaction with the gene expression, has been studied in a few plants *Arabidopsis thaliana* L., *Sesamum indicum* L., *Spinacia oleracea* L., *Triticum aestivum* L., *Solanum lycopersicum* L., *Cucurbita pepo* L., *Allium cepa* L., *Glycine max* L., *Nicotiana tabacum* L., *Oryza sativa* L., and *Hyoscyamus niger* L.

Some NMs like CeO_2 and Ag NPs produced genotoxicity in soybean and onion. The study demonstrated that they were detected in seeds, seedlings, and plants. In adult plants, they were present in the vascular system and leaves, suggesting uptake and translocation of the nanomaterial, respectively. Also, ZnO NPs (1, 10, and 50 μgm/L) damaged both root epidermal and cortical cells and significantly reduced *Lolium perenne* L. (ryegrass) growth. Besides, 10 mg/L or lower rates of these NPs did not influence root length (Khan et al. 2019a, b; Khan 2020). Acclimation to stress conditions and flexibility of the plant phenotype is related to the presence of genes that respond to stress conditions and genes that aid plant survival (Jordan et al. 2018). Like eukaryotes, the plant genome is shaped by interactions between histone proteins that regulate chromatin structure and DNA. In tests conducted, it could be observed that genetic changes due to DNA mutations, changes in DNA methylation pattern, histone modifications, and RNA interference play a key role in regulating plant genes (Khan et al. 2019a, b; Khan 2020; Khanna et al. 2015).

Studies have shown that epigenetic alterations in plants are responsible for controlling several aspects of their life cycle, including growth and development, fruiting time, and flowering. On the other hand, NMs cause epigenetic changes in DNA methylation and histone modification, including carbon-based NMs. Global acetylation and deacetylation are involved in plant responses to environmental stresses. The study of Yan et al. (2013) analyzed dynamic changes in histone acetylation during maize seedlings following SWCNT treatment. Maize seeds were germinated and treated with SWCNTs (20 mg/L). Chromatin immune staining in situ detection for root cells prepared at various time points using an antibody to acetylated H3 at lysine 9 (H3K9ac) was performed. Immunostaining using only secondary antibodies showed no specific labeling of nuclei. The results exposed that SWCNTs inhibited histone H3K9 acetylation. The expression levels of acetylated H3 at lysine 9 in cells were gradually increased as the extension of seed imbibition, and SWCNTs suppressed such an increase in global acetylation of H3K9 (Yan et al. 2013).

The hypermethylation occurred in the presence of MWNTs, which could guide the plants to respond to stress, as hypermethylation can also occur in plants responding to drought stress (Pagano et al. 2016). The up-regulation of abiotic stress-responsive genes following exposure to carbon-based NMs is related to the plant species. In tomatoes, this phenomenon has been studied in response to MWNT exposure. Fullerene soot (FS) showed a similar influence on *Arabidopsis thaliana* L. since defense genes involved in abiotic and biotic stress were up-regulated (Jordan et al. 2018). Additionally, up-regulation of the genes involved in defense and wounding responses elicited by foreign body or injury indicate mechanical damage of roots upon exposure to FS.

The roots of *Allium cepa* L., *Glycine max* L., and *Nicotiana tabacum* L. were exposed to NMs composed of Ag, ZnO, CeO_2, and TiO_2. It is known that these NMs altered the root meristem of several plant species when these materials were applied on. Correspondingly, the assays were random amplified polymorphic DNA and tests with comet and DNA laddering techniques (Chichiriccò and Poma 2015). The inhibition of the growth of the roots of treated plants was related to molecular phenomena of cell division and DNA damage. Also, DNA alterations were observed in *Zea may*s L. treated with TiO_2 reported by (Chichiriccò and Poma 2015). *Solanum lycopersicum* L. was exposed to carbon nanotubes (CNT), which resulted in the downregulation of genes associated with root and leaves and upregulation of genes associated with stress and water channels (LeAqp2). In a new study, the exposure of MWCNT resulted in upregulation of marker genes NNtPIP1, NtLRX1, and CycB, which are involved in the formation of the cell wall and cell divisions (Khodakovskaya et al. 2012; Kumar et al. 2019).

7.6 Proteins Damage

One of the alterations that have given a wide field of study is oxidative stress, which causes plants to produce reactive oxygen species (ROS) that can damage cell components and impair their functions. Plants continually encounter conditions that produce ROS, which is removed by a plant antioxidant system involving enzymes, proteins, and other metabolites that maintain cellular homeostasis (Kohan-Baghkheirati and Geisler-Lee 2015; Stadtman and Levine 2000).

The interaction between NP and is mediated by various mechanisms that can be chemical or physical. ROS production, cell membrane ion transport disruption, lipid peroxidation, or oxidative damage all correspond to chemical interactions. The preceding given that the NM to enter the plant cells act as metal ions, reacting with the sulfhydryl and carboxyl groups, altering the activity of the proteins. In general, oxidative stress and ROS production cause modification in proteins called protein oxidation (protein oxidation) (Stadtman and Levine 2000).

Oxidation in proteins is generally measured with the levels of protein carbonyl increase because an increase or decrease in proteins is the first response of the plant to oxidative stress. However, this does not always occur. Plants can also accumulate or synthesize types of proteins such as heat shock proteins (HSPs) (Zhao et al. 2016, 2012), Zinc-finger proteins (Takatsuji 1999), mitogen-activated protein kinase (MAPK) (Cristina et al. 2010), among others. Nevertheless, they are still one of the main targets of ROS. The phytotoxicity of NMs has been widely evaluated at the morphological, physiological, and biochemical levels. However, few studies have given importance to the modification at the proteomic level, even though proteins are essential in all living cells as they have several functions such as cell signaling, regulation, catalysis, intra- and intercellular movement of nutrients and other molecules, membrane fusion, support, and structural protection (Anjum et al. 2015). Their function will depend on their structure, acquired after ribosomal synthesis of their amino acid chain. In addition, the conformation of a protein will depend on the physical and chemical conditions of the protein environment, which is easily affected by extreme temperatures, reactive molecules, metal ions, and the presence of foreign agents such as NMs that disrupt the folding process of a newly synthesized protein giving incorrect folds (Hasan et al. 2017).

Vannini et al. (2014) observed the molecular response of exposing wheat seeds with commercial 10 mm Ag NPs coated with PVP (Polyvinyl pyrrolidone) using a concentration of 10 mg/L for all experiments. No effect on seed germination speed was observed, and proteomic analysis confirmed that roots are the main targets of toxic effects; roots and shoots were analyzed. Several proteins involved in primary metabolism were detected, confirming that metabolic adaptation of the plant is essential when exposed to external agents.

In the roots, the presence of three α-amylases and a fructose bisphosphate aldolase was observed, confirming that there was a lower accumulation of amyloplasts in root cells exposed to Ag NPs. In Ag NPs-treated shoots, increased aconitate hydratases were observed, indicating that enzymes were produced that help regulate oxidative stress caused by the NPs. The negative impact of NPs on shoots is also confirmed by the downregulation of HCF136 protein, which is essential for biogenesis (Vannini et al. 2014).

Tripathi et al. (2017a, b, c) observed the effect of Ag NPs, at concentrations of 1000 and 3000 μM on Aloe vera and *Pisum sativum* L. plant, to follow the effect of protein oxidation follows the production of antioxidative enzymes using the method of Giannopolitis and Reis (1977). They conclude that enzyme production was low, so it had an inadequate response to the presence of Ag NPs, which resulted in a decrease in plant size due to the stress produced by the NPs (Tripathi et al. 2017a, b, c; Singh et al. 2017).

Salama (2012), made a study of how the main characteristics change among the carbohydrate and protein content of economically important plants such as common bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.), using five concentrations of silver nanoparticles (20, 40, 60, 60, 80 and 100 mg/L). It was observed that low concentrations (20 to 60 mg/L) of Ag NPs had a stimulating effect on crop growth and increased the concentration of proteins and carbohydrates but increasing the concentration of Ag NPs reduced these compounds (Salama 2012).

Tang et al. (2016), performed a microarray analysis of toxicogenomic effects of CuO in *Arabidopsis thaliana* L. The experiment was carried out using two concentrations of 10 and 20 mg/L of CuO. At the same time, the experiment was done using Cu^{2+} ions, for these ions were used concentrations of 0.8 and 1. 35 mg/L. The toxicogenomic assay was done, and this showed that out of 1035 genes, 603 were up or downregulated. The 47 positively altered genes were analyzed using real-time PCR and agreed with the microarray analysis. It was observed that the upregulation of genes involved in response to stress response when NPs were used (Tang et al. 2016).

Mirzajani et al. (2014), carried out a study with Ag NPs to understand the population and subpopulation of proteins and track environmental stresses. They exposed *Oryza Sativa* L. to a colloidal solution of spherical Ag NPs. The proteomic analysis revealed that 28 Ag NPs-sensitive proteins were identified (decrease and increase in excess). The proteins identified in both root and plant are related to oxidative stress tolerance. Direct DNA/RNA/protein damage at the cellular level, cell division, transcription and protein degradation, and apoptosis, showing the interaction of NPs with cell metabolic processes such as synthesis/degradation and apoptosis (Mirzajani et al. 2013, 2014).

Vannini et al. (2014), observed that the part that suffered the most damage when Ag NPs were present were the roots´ plants. Differentially expressed proteins were found in roots and shoots, which may be related to differences in the amount of Ag accumulated in these two organs. The accumulation of the enzymes, three α-amylases, and one fructose bisphosphate aldolase agree with the observation that there was a lower amount of amyloplasts in root cells exposed to Ag NPs, in shoots increased levels of aconitate hydratases. The alteration of stress levels by Ag NPs can translate into a decrease in plant size (Vannini et al. 2014).

Rico et al. (2014), observed the growth of wheat plants in soils previously treated with cerium oxide NPs (CeO_2) at concentrations of 0, 125, 250, and 500 mg/Kg and showed the effect on growth, yield, and nutritional value of wheat (*Tricum aestivum* L.). Modifications were observed in the roots but not in the leaves, husk, and grains, which speaks of poor transport of Ce to the rest of the plant. As a finding, they identified that CeO_2 interfered with increasing amino acid (AA) content in wheat grains. There is no other study on this effect with nanomaterials, so it makes its interpretation challenging. However, the studies proved that AA metabolism or protein content was modified in wheat and rice, while several NPs modified protein levels (Rico et al. 2013a, b; Rico et al. 2014).

Yasmeen et al. (2017), show a proteomic study to see how it affected Cu and Fe NPs in wheat plants. 25 mg/L of Cu and Fe NPs were used, and free-gel proteomic analysis was done, finding total changes in abundance of 58, 121, and 25 proteins in wheat seeds of three species Galaxy-13, Pakistan-13, and NARC-11. Then an increase of 25 mg/L of Cu NPs was done, increasing SOD activity, suggesting that Cu NPs improve stress tolerance of wheat (Hossain et al. 2020; Yasmeen et al. 2018; Yasmeen et al. 2017).

Rani et al. (2016) evaluated the use of Ag NPs synthesized chemically and biologically (S–Ag NPs and B-Ag NPs) on water hyacinth (*Eichhornia crassipes* L.). NPs were placed in concentrations of 1, 10, and 100 mg/L in the water where the plants were placed, and their growth was observed for five days, a decrease in the plant's growth treated with S–Ag NPs. The biochemical analysis shows an increase in carbohydrates and proteins in the plants treated with B-Ag NPs and a decrease in the production of chlorophyll and phenol. In contrast, those treated with S–Ag NPs showed no change in the number of proteins and carbohydrates but a significant increase in the amount of phenol and chlorophyll. An increase in antioxidant enzyme activity was observed in S–Ag NPs-treated plants, and no such change was seen in B-Ag NPs (Rani et al. 2016).

Zhao et al. (2012), studied the effect of adding CeO_2 NPs in the soil where maize plants were grown; concentrations of 400 and 800 mg/Kg of CeO_2 NPs were used. It was analyzed parameters related to oxidative stress such as H_2O_2, CAT, ascorbate peroxidase activity (APX), lipid peroxidation, and heat shock protein 70 (HSP70). The analyses concluded a stress response in the plant by producing HSP70 and H_2O_2, but there was no loss of membrane integrity, thus demonstrating that maize plants have a protective response to CeO_2 particles (Zhao et al. 2012, 2013).

Hossain et al. (2016), compared the phytotoxicity of Al_2O_3, ZnO, and Ag NPs on soybean seedlings at the proteome level. They observed that Ag NPs, ZnO produced significant root stiffness, plant growth, and root cell viability changes. Also, a considerable oxidative stress gel-free proteomic analysis showed 104 changed proteins associated with metabolism, hormone metabolism, and cellular organization. Plants treated with Al_2O_3 maintained average growth and were favored with abundant proteins involved in oxidation–reduction, growth-related hormonal pathways and could be a key to optimal soybean growth under stress in the presence of Al_2O_3 (Hossain et al. 2016, 2020).

The effect of using Cu NPs at concentrations of 1, 5, 10, and 50 mg/L on Pakistan-13 and NARC-11 species of wheat using proteomic technique increasing metabolism-related proteins in Pakistan-13 species and NARC-11 species, increases in photosynthesis-related proteins were observed. In contrast, glycolysis and the tricarboxylic acid cycle proteins increased with exposure to NPs in both species. The plant was benefited in growth when 10 mg/L Cu NPs were used (Yasmeen et al. 2017).

Mustafa et al. (2016) studied the effect of Ag NPs size on soybean seeds, using three sizes of NPs 10, 15, and 50–80 nm at a concentration of 5 mg/L. Morphological analysis revealed that Ag NPs with a size of 15 nm promoted soybean growth under flooded conditions. An increase of 228 proteins was observed under flooding conditions with and without pin the presence of small Ag NPs. A decrease in the number of proteins related to protein synthesis was observed compared to the control group. In contrast, the number of proteins related to amino acid synthesis and ribosomal proteins was increased in the presence of 15 nm Ag NPs, so it was concluded that the size of the nanoparticle might affect soybean growth by producing proteins when under stress (Hashimoto et al. 2020; Hossain et al. 2016; Mustafa et al. 2016).

Hashimoto et al. (2020), performed a gel-free proteomic study to analyze the interaction of Ag NPs with nicotinic acid and potassium nitrate (KNO_3) (5 mg/L Ag NPs, 8 μM nicotinic acid, and 0.1 mM HNO_3) with soybean hypocotyl (*Glycine max* L.). Out of 6340 identified proteins, 351 presented significant changes, 247 increased, and 104 decreased. These proteins were related to protein degradation and synthesis. Cell death was higher in soybean roots. Soybean seedlings exposed to Ag NPs, nicotinic acid, and KNO_3 improved under flooding (subjected to stress), suggesting that the mixture positively affects soybean seedlings by regulating protein quality control and improving growth under flooding (Hashimoto et al. 2020).

7.7 DNA Damage

There are two known mechanisms involved in DNA damage produced by nanoparticles. By direct physical contact, specifically, this interaction is carried out between the nitrogenous bases or breaking chromosomes. These actions impair basic processes of DNA replication by forming stable molecular adducts or by changing the regular gene expression pattern (Singh et al. 2017).

The indirect damage produced in the DNA is related to the interaction of the whole proteins associated with the normal DNA function or by disturbing cellular mechanisms involved to maintain the cellular homeostasis. A clear example is an interference with proteins involved in the DNA replication-cell division process, the synthesis of ROS, and the endogenous ROS detoxification systems (Mehrian and De Lima 2016). This genotoxic capacity of nanoparticles depends on several factors, such as the size of the nanoparticle and its concentration. It is worth considering that the DNA damage is not uniform in the whole plant, considering their size and different cell compartments (Heikal et al. 2020; Kokina et al. 2015). The genotoxic effect of metallic and metallic oxides NMs has been investigated. The effect of ZnO of different sizes was studied in *Allium cepa* L. (onion), *Nicotiana tabacum* L. (tobacco), and *Vicia faba* L. (bean) at a concentration of 0.4 gr/L. This nanomaterial shows an increment in DNA damage due to genome fragmentations micronuclei. Then is possible to conduct cell cycle arrest and even cell death (Ghosh et al. 2016).

Also, the nanoparticles produced by green synthesis using water extracts of the plant *Eichhornia crassipess* L. (water hyacinth) can induce several abnormalities in *Allium cepa* L. On this plant was observed a process called citomixis. Also, nuclei morphological changes were developed, and micronuclei formation was observed. Finally, an interesting effect was detected since the standard chromatin structure was missing; considering the importance of this epigenetic regulation, more research must be conducted on this topic (Heikal et al. 2020). The effect of ZnO produced either by chemical synthesis or green synthesis using latex from *Calotropis gigantea* L. was analyzed about the genotoxic effect on the plant *Lathyrus sativus* L. Similar damage was observed using both nanoparticles, analyzed by the comet test to observe DNA damage (Panda et al. 2017). The DNA damage produced by nanoparticles is also related to the oxidative stress produced by these materials. In an experiment conducted in celery (*Coriandrum sativum* L.) using Cu NPs at a concentration of 200 mg/L to 800 mg/L of Cu NPs, a high increase in the H_2O_2 was measured either in leaves or roots. This oxidative burst can induce DNA mutations like deletions (AlQuraidi et al. 2019). On the other hand, experiments conducted on eggplant (*Solanum melongena* L.) show the degree of DNA damage concerning the progression of the cell cycle using nanoparticles of cobalt oxide (Co_3O_4), a high increase in ROS production was detected even at low concentration of this nanomaterial (0.25–1 mg/ml).

DNA fragmentation was observed, and a similar effect was obtained when TiO_2 or ZnO nanoparticles were used (Faisal et al. 2016, 2013). In the bryophyte plant *Psyscpcomitrella patens*, a treatment with manganese oxide nanoparticles (20 μg/ml) induces the synthesis of superoxide anion and H_2O_2. Also, the genotoxicity as DNA fragmentation was observed at a concentration as low as 10 μg/ml. The degree of DNA hypomethylation in these experiments was also analyzed, and it is essential to consider the impact of the methylation pattern with DNA epigenetic effects (Ghosh et al. 2019). In tomato (*Solanum lycopersicum* L.), the DNA stability is reduced by Ag NPs; this is related to the increase in the oxidative stress produced by the nanomaterial. A reduction in the endogenous antioxidant system was also detected (ÇekİÇ et al. 2017). Besides the induction of DNA fragmentation, mutations due to oxidative stress also chromosome anomalies are detected in plants under treatment of metallic nanoparticles. The mitotic rate was drastically diminished in lentils (*Lens culinaris* Medik.) treated with TiO_2. An increase in the chromosome abnormalities like sticky chromosomes at metaphase, fragmentation during anaphase, a lagged telophase, and micronuclei formation was evidenced due to this material (Khan et al. 2019a, b). The size of the particle is an essential feature to consider about the genotoxic property of the nanomaterials since a more dramatic genotoxic effect was observed in nanoparticles smaller than 10 nm, compared with bigger particles (Ghosh et al. 2016; Kokina et al. 2015). The small size is related to their solubility, reactivity, and interaction with several cellular compounds, either in animals or plants cells. Usually, the nanoparticles around 10–15 nm in size are stuck on the cell membrane. Then it is more feasible than the small size particles damage the DNA damage, due to this small size can reach the gene DNA compartment (Kokina et al. 2015). The effect of copper nanoparticles was studied in cucumber (*Cucumis sativus* L.) using 10–30 nm particles. The cucumber DNA was analyzed by random DNA amplification of polymorphic DNA; the analysis of this material revealed mutations on the genome (Mosa et al. 2018).

7.8 Plant Responses Towards NMs Toxicity

The seeds germination and its mechanism are of great importance where it is mainly categorized by the absorption of water, the lag phase, and the exit of the root. It has been observed that nanoparticles enter the seed during water absorption, although they can also be absorbed on the surface of the seed coat (Campaña and Arias 2020; Das et al. 2019).

Its transportation occurs through empty spaces present in parenchymal tissues, and its movement is across the cotyledons. The facile absorption in the seed can have both positive and negative influences. It is known that the nanoparticles uptake by the plant is through an active transport mechanism over the xylem where the nanoparticles are taken from the soil by the root of the plants. This transport is dependent on the size, the chemical interactions with molecules of the cell membranes, and the reactivity of the nanoparticles in the cytoplasm. That will produce different metabolic or structural biochemical pathways of the plant, which will be noticed in seed germination, seedling sprouting, radicle and plumule length, weight, enzymatic activities, antioxidants, and essential physiological processes such as photosynthesis and respiration (Campaña and Arias 2020; Das et al. 2019; Lin and Xing 2007).

ROS production is the by-product of normal metabolic pathways that occur in cell organelles. However, under stress conditions, excess oxidation can be caused throughout electrons transference generating oxidative stress causing direct damage to the cell membrane permeability, proteins, and DNA, ultimately generating cell death (Anjum et al. 2015, 2019; Bagherzadeh Homaee and Ehsanpour 2016; Cabiscol et al. 2000; Faisal et al. 2013; Farooq et al. 2019; Jeevanandam et al. 2018; Mirzajani et al. 2013; Poborilova et al. 2013; Stadtman and Levine 2000; Tan et al. 2009; Tang et al. 2016; Zhao et al. 2016; Zulfiqar and Ashraf 2021). Generation of ROS or free radicals is one of the mechanisms that nanoparticles cause, forming more significant oxidative stress, lipidic peroxidation, and therefore inflammation of the cell.

The oxidative stress can damage proteins and membranes, organelles, and DNA cells, generating necrosis of cellular structure, damage of the DNA membrane, growth retardation, lethality, autoimmunity, and the loss of cellular functions in the plant. The cytotoxic effects of nanoparticles affect cell morphology, mitochondrial function, plasma membrane permeability, and apoptosis (Stampoulis et al. 2009; Tripathi et al. 2017a, b, c). In the case of soils with bacterial communities, it has been reported that titanium oxide and zinc oxide nanoparticles have altered essential soil processes. However, a direct connection has not been found concerning particle size, but metal ions, metal oxides, and sulfates, which biochemically disturb bacteria (Zhao et al. 2016).

First, to establish a nanomaterial as a toxic one, some properties must be considered, such as solubility in a specific material solvent that can impact the bioavailability, the pH interval or physical state, susceptibility to form aggregates or agglomerates. ROS generation makes them able to act as carriers of toxic substances. However, it has been observed that the coating of the nanoparticles can minimize toxicity and the functionalization of some materials such as carbon nanotubes or silver nanoparticles. With coatings of organic or inorganic compounds to cover the surface and avoid aggregation like citrate, polyvinylpyrrolidone (PVP), and cetyltrimethylammonium bromide (CTAB) have restricted their cytotoxicity (Cvjetko et al. 2017, 2018; Messa and Faez 2020). The germination is not favorable, and it is believed that there may be a risk impact of nanomaterials both in humans and other life forms due to this uncontrollable addition. In general, the negative impacts of these nanomaterials have been seen primarily on the production of crops and harmful consequences in the ecosystem because they harm the microbial system of the soil, the availability of nutrients for arthropods and annelids, the soil inhabitants (Das et al. 2019; Dasgupta et al. 2017; Sardoiwala et al. 2018).

The challenge is the correct determination of the number of nanomaterials applied to avoid impacts on the environment; therefore, toxicological research and the behavior of nanomaterials in the agricultural sector should be mandatory (Agathokleous et al. 2019; Das et al. 2019; Jeevanandam et al. 2018; Yan and Chen 2019). The nanoparticles concentration in seeds has been correlated with the toxicity of plants. At a high amount, an impact on germination has been observed and on root growth. However, at low doses of nanoparticles, the impact is negligible. The toxicity of the nanoparticles also affects the characteristics of the seed coatings regarding their thickness and chemical composition. The most pronounced effect has been the reduction in the germination index concerning the concentration and sizes of the nanoparticles, since below 100 nm nanoparticles can cross biological membranes with ease, observing it mainly in nanoparticles of Silicon (Si), Silver (Ag), Gold (Au), iron (Fe), copper oxide (CuO), zinc oxide (ZnO), titanium dioxide (TiO_2), single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), C_{60} fullerenes, Quantum Dots (QD), and virus-based nanoparticles (VNPs). Exposure to high concentrations of nanoparticles above the threshold has adverse effects on plants (Ruttkay-Nedecky et al. 2017).

The development of some consequences by nanoparticles both in plants and soils depends mainly on the physicochemical properties of nanoparticles, the conditions of the crop, and the type of plant species. It has been observed that they can generate positive and negative effects. One of the most significant situations is that nanoparticles can change their structure and ionization state, which form complexes with other molecules or nutrients and even accumulate in such a way that they can negatively affect plants (López-Moreno et al. 2018). The effects of nanoparticles in some cellular processes have been observed in their accumulation in the cellular system since it is not capable of breaking them, which causes a rupture of the DNA in the plant system and, therefore, the modification in the genome. However, more research is needed concerning these issues. Cells are affected by the modulation of cellular functions through various action mechanisms of nanomaterials such as the generation of oxidative stress, inflammation in cell membranes and organelles, and the generated genotoxicity due to the exposure of nanomaterials in viable cells (Das et al. 2019). Statistical studies have suggested that the addition of nanomaterials in seeds produces effective responses with particles with sizes greater than or equal to 100 nm. However, other parameters must be inquired in the toxicology of these nanoparticles, such as particle size, aggregation, hetero-aggregation, shape, surface functionalization, crystallinity, metal concentration, and stabilizers (Agathokleous et al. 2019; Tripathi et al. 2017a; b, c).

To analyze the toxicity, the main parameters observed in the plant are the growth potential, seed germination percentage, biomass accumulation, and the leaf's surface area. The most common symptoms are the obstruction of the pores and barriers in the apoplastic current, reduction of photosynthetic processes, generation of ROS, and damage to DNA structures, in addition to many severe anatomical and morphological changes (Ghosh et al. 2010; Panda et al. 2011; Shen et al. 2010; Vannini et al. 2014; Vishwakarma et al. 2017). Some investigations have shown both positive and negative effects in the application of nanoparticles in plants to morphological, physiological, cellular, and molecular levels (Das et al. 2019; Hasan et al. 2017; Jacob et al. 2013; Ruttkay-Nedecky et al. 2017; Song et al. 2013). As mentioned, NMs of different compounds have been used in agriculture, finding different plant behaviors (see Table 7.3).

The mechanism for the uptake and translocation of nanoparticles in plants depends on the type of plant and how the nanoparticles are added. In the case of the addition of the nanoparticles by the root, they penetrate the cell wall and the plasma membranes of the epidermal layers in the roots. However, studies have been carried out where the application of the nanoparticles is foliar, this thorough immersion or spray application, which is not more effective, has been observed that when the leaves are exposed to the nanoparticles, they accumulate in the stomata instead of the vascular beam and finally move to different parts of the plant through the phloem, the accumulation is higher than when the nanoparticles are added by the roots (Chhipa 2019; Miralles et al. 2012; Raliya et al. 2016; Tripathi et al. 2017a, b, c).

One of the most critical matters of the toxicity of nanoparticles is that they can accumulate in different areas of the plant. Once the nanoparticles enter the tissues, the transport directs to seeds and fruits, generating total contamination where human life is involved (Yan and Chen 2019). The interaction among plants and nanoparticles is complicated and is influenced by different parameters such as plant species and tissues, the intrinsic properties of nanoparticles such as size, concentration, and surface coating. However, it has been concluded that not all plants treated with nanoparticles exhibit toxic effects (Chhipa 2019; Hashimoto et al. 2020; Hossain et al. 2016; Jeyasubramanian et al. 2016; Liu and Lal 2015; Mustafa et al. 2016; Pradhan and Mailapalli 2020; Pradhan et al. 2013; Wang et al. 2016).

Table 7.3 Positive and negative effects in plants of NMs to morphological, physiological cellular, and molecular levels

NMs	Effect	Plant	References
SiO_2	Enhanced germination rate, germination of seeds, and upgrowth percentage Nanoparticles were present in the intercellular space of leaves following vacuum infiltration	*Lycopersicon esculentum* L *Zea mays* L *Arabdiopsis thaliana* L *Triticum aestevium L* *Lupinus luteus* L	Siddiqui and Al-Whaibi (2014) Suriyaprabha et al. (2012) Hussain et al. (2013) Hussain et al. (2013) Hussain et al. (2013)
TiO_2	Differences in root growth located enhanced lycopene content Favors the growth of leaves, reduces the number of lateral roots in pea, blocks transpiration, and declines hydraulic conductivities Generation of ROS, causing lipidic peroxidation, oxidative stress in chloroplasts, altered chlorophyll content, and the modification of activities of enzymatic antioxidants Caused elimination of microtubule, damage to the genomic DNA, increased tubulin monomers that ultimately affect the proteasome system causes chromosomal aberrations through fragmentation of arms of chromosomes	*Solanum lycopersicum* L *Cucumis sativus* L *Spinacia oleracea* L *Pisum sativum* L *Zea mays L* *Phaseolus vulgaris* L *Arabidopsis thaliana* L *Cucurbita pepo* L *Vicia narbonensis* L	Raliya et al. (2015a, b) Servin et al. (2013) Lei et al. (2008) Fan et al. (2014) Asli and Neumann, (2009), Ruffini Castiglione et al. (2011) Jacob et al. (2013) Wang et al. (2011) Moreno-Olivas et al. (2014) Castiglione et al. 2011

(continued)

Table 7.3 (continued)

NMs	Effect	Plant	References
FeO	Increased root, stem, plant length, and biomass Increase chlorophyll levels The α-amylase activity was increased and did not induce oxidative stress in the plant It reduces the root hydraulic conductivity (Lo) had a significant influence on the production of hormones: abscisic acid (ABA) and indole-3-acetic acid (IAA)	*Oryza sativa* L *Arachis hypogaea* L *Vigna radiata* L *Spinacea oleracea* L *Lactuca sativa* L *Helianthus annuus* L *Glycine max* L *Solanum lycopersicum* L	Gui et al. (2015) Rui et al. (2016) Raju et al. (2016) Jeyasubramanian et al. (2016) Trujillo-Reyes et al. (2014) Martínez-Fernández et al., (2016) Alidoust and Isoda (2013), Ghafariyan et al. (2013), Yang et al. (2020) Martínez-Fernández and Komárek (2016)
Fe_3O_4 coated with citric acid	It increased root and shoot weights It increased chlorophyll content Increased catalase activity in roots	*Glycine max* L *Medicago sativa* L	Iannone et al. (2021)
ZnO	Improved germination Increase in stem height and root length Cause oxidative stress decreased chlorophyll content Reduce and collapse the size of the vacuole, cortical cells, epidermis, and roots, generating a reduction of the cell turgidity and size of the cell Obstruction in plasmodesmata Cause obstruction in the process of translocation	*Zea mays L* *Solanum melongena* L *Brassica oleracea var. capitata L* *Solanum lycopersicum* L *Vigna radiata* L *Triticum aestivum* L *Vigna unguiculata* L *Cucumis sativus* L *Pisum sativum* L *Abelmoschus esculentus* L	Adhikari et al. (2016), Pokhrel and Dubey (2013) Baskar et al. (2018) Pokhrel and Dubey (2013) Raliya et al. (2015a, b) Raliya et al. (2016) Savi et al. (2015) Wang et al. (2013a, b) Zhao et al. (2013) Das et al. (2019)

(continued)

Table 7.3 (continued)

NMs	Effect	Plant	References
Al_2O_3	It caused a reduction in enzymes activities Increase in root size Increase nutrient uptake	*Tobacco BY-2 cell* *Lactuca sativa* L	Poborilova et al. (2013), Poborilova et al. (2013) Hayes et al. (2020)
CuO	Favorable growth and product quality Oxidative damage in DNA plant Decrease in seed germination, root length, root biomass, water content, dry biomass of the plant Bioaccumulation in roots and leaf cells	*Rapanus sativus* L *Solanum lycopersicum* L *Lactuca sativa* L *Cucumis sativus* L *Lolium perenne* L *Lolium rigidum* L *Elsholtzia splendens* Nakai ex Maekawa *Zea mays* L *Oryza sativa* L *Abelmoschus esculentus L*	Atha et al. (2012) Juárez-Maldonado et al. (2016) Trujillo-Reyes et al. (2014) Zhao et al. (2016) Liu and Lal (2015) Liu and Lal (2015) Shi et al. (2014) Yang et al. (2015) Yang et al. (2015) Liu and Lal (2015)
NiO	Decrease in biomass Repression in the growth of shoots and roots It increased ROS generation Decrease in chlorophyll Alteration in phenolic and total flavonoid contents It reduced radical scavenging activity	*Abelmoschus esculentus* L	Baskar et al. (2018)
Y_2O_3	Seed germination was delayed at high concentrations (50 and 100 mg/L) At concentrations higher than 20 mg/L, root elongation was inhibited, and decrease in chlorophyll production and a significant reduction in Chlorophyll content NPs accumulated mainly in the roots	*Oryza sativa* L	Zhao et al. (2021)

7.8.1 Conclusions and Prospects

Due to the increasing demand for food production needed to feed humanity is imperative the implementation of new technologies to meet our food production targets. Nanotechnology, particularly nanomaterials, is a feasible alternative to implement in the agricultural sector. Nevertheless, as a new and emerging technology, several concerns firstly must be addressed, e.g., their toxic potential after introduction to the trophic chain. Also, their beneficial effects must be considered as inputs in agricultural production. Some of the NMs tested are metal-based metal oxide-based and carbon-based materials. It is undeniable that NMs exert their toxic effect due to the highly oxidative molecules production, which negatively impacts DNA or membranes, impairing basic cellular processes. However, plant cells can counteract oxidative stress by enzymatic or non-enzymatic mechanisms, mainly avoiding the mutagenic effects on DNA and proteins. Some NMs induced plants to develop more radicular biomass, which will impact the amount of nutrients uptake and water. This will be reflected on stronger stems that support the fruits better, and some NMs improve leaf area development. Therefore, the amount of chlorophyll is higher, but we always have to consider the plant species since specific responses have been demonstrated. Therefore, the positive and negative effects of NMs must be evaluated to establish protocols to guarantee their safety and benefits to humankind and environment.

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Part II
Cellular Macromolecules

Chapter 8
Interaction of Nanoparticles with Plant Macromolecules: Carbohydrates and Lipids

Lei Qiao and Chunlan Xu

Abstract Nanotechnology is proposed to improve plant growth and meet the global demand for food as a result of a rapidly increasing world population. Nanoparticles (NPs) are regarded as a feasible strategy for the implementation of sustainable agriculture due to their unique chemical and physical properties, including high reactivity, multi-biological activities, high surface area, and tunable particle size. Many NPs have received considerable attention due to their ability to enhance the growth and yield of various plants. NPs are able to stimulate plant development via positive effects on promoting plant seed germination, plant root or above-ground growth improving stress tolerance, which is closely associated with plant carbohydrates and lipids. Many studies have confirmed that NPs can promote the synthesis of carbohydrates and inhibit lipid peroxidation, thereby improving plant yield and stress resistance. Furthermore, biosynthetic NPs are an effective strategy to replace traditional NPs and agrochemicals. Compared to physical and chemical methods, plant macromolecules, especially carbohydrates, provide an environment-friendly, safe and efficient method for the synthesis of NPs. Biogenic NPs synthesized by plant carbohydrates also have been widely applied in agriculture to stimulate plant growth. This review summarizes the effects of the different NPs on plant carbohydrates and lipids and the green synthesis of NPs using plant carbohydrates.

Keywords Biosynthesis · Carbohydrates · Lipids · Nanoparticles · Plant

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8.1 Introduction

Nanotechnology is a dynamic research and innovation field, which affects our daily life in many ways. Nanoparticles (NPs) are materials with the size of 1–100 nm and have unique biological, chemical and physical properties. NPs have many characteristics that traditional large-size materials do not have, such as surface and interface effect, small-size effect, and quantum tunnel effect making them widely used in biomedicine, agriculture, and the environment (Xu et al. 2019; Khan et al. 2021). To achieve as much as possible to obtain the largest agricultural output from the existing resources, and to alleviate the pressure caused by the increasing shortage of global resources and the growing population, many NPs are continuously being used in agriculture (Shang et al. 2019). NPs are able to stimulate plant development via positive effects on promoting plant seed germination, plant root or above-ground growth improving stress tolerance, which is closely associated with plant carbohydrates and lipids (Zhao et al. 2020; Josef et al. 2021; Wang et al. 2020). Carbohydrates and lipids are the main components of plants. Studies have shown that NPs can promote the synthesis of carbohydrates and inhibit lipid peroxidation to increase plant yield (Siddiqui et al. 2015; Zhao et al. 2020). In addition, the green synthesis of NPs via environment-friendly plant macromolecules, such as carbohydrates, has a significant potential to boost NPs production (Xu et al. 2021). In recent years, biogenic NPs synthesized by plant carbohydrates also have been widely applied in agriculture to stimulate plant growth (Landry et al. 2019; Kah et al. 2019). The present chapter summarizes recent advances in the application of different NPs to promote the synthesis and accumulation of plant carbohydrates and inhibit lipid peroxidation (Fig. 8.1), and the use of plant carbohydrates for the green synthesis of NPs.

8.2 Effects of Nanoparticles on Plant Carbohydrates and Lipids

The positive role of NPs in the environment, especially in plant ecosystems, has been extensively studied. Many NPs have received considerable attention due to their ability to enhance the growth and yield of various plants (Mittal et al. 2020). The main purpose of this section is to collect information on the positive effects of different NPs in plant growth and increase production, especially to promote the synthesis of carbohydrates and inhibit lipid peroxidation (Table 8.1).

8.2.1 Silver Nanoparticles

Silver nanoparticles (AgNPs) are widely used in coatings, medical fields, environmental remediation, textiles, and other fields because of their strong antibacterial

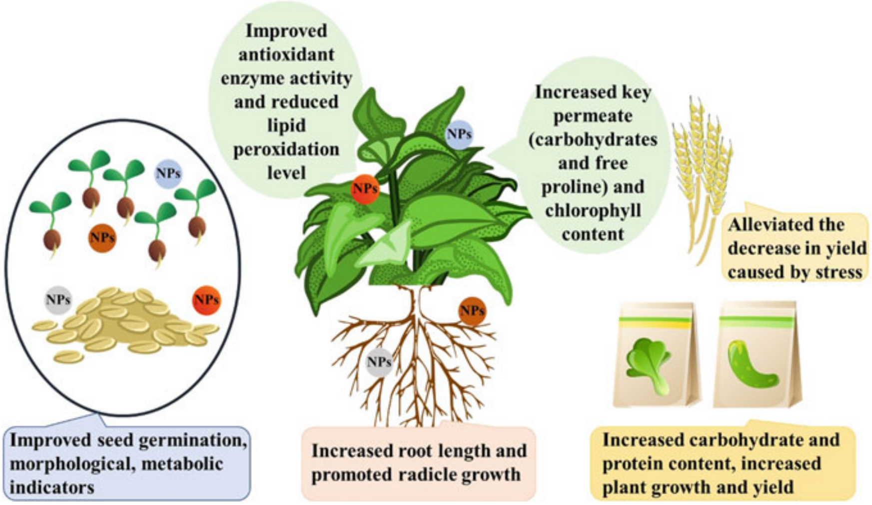

Fig. 8.1 The positive effects of nanoparticles (NPs) on seed germination, plant growth and yield. Graph by Lei Qiao

activity against different pathogenic microorganisms (Burdusel et al. 2018). In agricultural production, AgNPs have received great attention due to their ability to enhance the growth and yield of various crops (Mehmood 2018). Mehmood et al. (2017) described the effect of AgNPs prepared from *Berberis lycium* Royle root bark extract on field pea (*Pisum sativum* L.) seed metabolism. The results demonstrated that AgNPs-treated plants had higher carbohydrate contents, which led to better growth and yield of pea. In this study, the highest carbohydrate seed content of *Pisum sativum* L. after seed treatment and foliar spraying was recorded in response to the application of 60 ppm AgNPs. Sadak's (2019) work proved the effect of AgNPs on the fenugreek plant (*Trigonella foenum-graecum*), and the results indicated that treatment with AgNPs on the foliage of the fenugreek plant led to a significant increase in total carbohydrates compared to control plants and 40 mg/L AgNPs treatment made the carbohydrate content reach the maximum level. A study carried out by Latif et al. (2017) demonstrated that AgNPs (20 and 40 ppm) biosynthesized using *Mangifira indica* and *Ocimum basilicum* had significant effects on the growth of wheat (*Triticum aestivum*) seedlings, as revealed by the increases in dry and fresh weights, carbohydrate content, and chlorophyll content. Salama's (2012) research showed that after applying 60 ppm AgNPs, the carbohydrate content of *Zea mays* L. and *Phaseolus vulgaris* L. plants increased by 57% and 62%, respectively, compared with the control. A study by Krishnaraj and co-workers (2012) reported the beneficial effects of the synthesized AgNPs on *Bacopa monnieri* (Linn.) Wettst seed germination, which induced the synthesis of protein and carbohydrate, and decreased the levels of total phenol content, catalase (CAT), and peroxidase compared to the $AgNO_3$ treated plants. In addition, Gupta et al. (2018) found that the addition of of biosynthesized AgNPs into the medium enhanced rice (*Oryza sativa* L.) seedling growth (Fig. 8.2),

Table 8.1 Positive effects of different NPs plant carbohydrates and lipids

Nanoparticles	Plant	Responses	References
AgNPs	*Pisum sativum* L	Increased carbohydrate contents and enhanced growth and yield of pea	Mehmood et al. (2017)
AgNPs	*Trigonella foenum-graecum*	Improved the total carbohydrates	Sadak (2019)
AgNPs	*Triticum aestivum*	Increased the fresh and dry weight, chlorophyll and carbohydrate contents of seedlings	Latif et al. (2017)
AgNPs	*Phaseolus vulgaris* L *Zea mays* L	Improved the level of carbohydrates	Salama's (2012)
AgNPs	*Bacopa monnieri*	Enhanced germination and growth of seedlings, induced the synthesis of carbohydrate and protein, and reduced the levels of total phenol content, catalase (CAT), and peroxidase activities	Krishnaraj et al. (2012)
AgNPs	*Oryza sativa* L	Improved the growth of rice seedlings, decreased hydrogen peroxide (H_2O_2), reactive oxygen species (ROS) and lipid peroxidation levels	Gupta et al. (2018)
AgNPs	*Daucus carota L*	Increased activities of antioxidant enzymes and reduced the level of ROS and malondialdehyde (MDA)	Faiz et al. (2022)
AgNPs	*B. campestris L*	Accelerated seed germination speed and seedling development and increased cabbage yield	Zhou X et al. (2022)
SeNPs	*Fragaria × ananassa* Duch	Alleviated the adverse effects of soil salinity on the growth and yield of strawberry plants, increased the levels of total soluble carbohydrates and free proline reducing soil-salinity stress-induced lipid peroxidation and H_2O_2 content	Zahedi et al. (2019)

(continued)

Table 8.1 (continued)

Nanoparticles	Plant	Responses	References
SeNPs	*Capsicum annuum* L	Increased the levels of chlorophyll and soluble sugars	Li et al. (2020)
SeNPs	*Punica granatum* L	Reduced the lipid peroxidation and H_2O_2 content induced by drought stress by enhancing the activity and content of antioxidant enzymes	Zahedi et al. (2021)
SeNPs	*Brassica napus* L	Reduced the oxidative stress and membrane lipid damage caused by Cadmium (Cd)	Qi et al. (2021)
SeNPs	*Vicia faba*	Enhanced seed germination, morphological and metabolic indicators, and suppressed pathogen *Rhizoctonia solani*	Hashem et al. (2021)
SeNPs	*Coriandrum sativum* L	Alleviated Cd toxicity through enriching chlorophyll content, total soluble sugars, leaf relative water content, improving gas exchange parameters and modulating the antioxidant system	Sardar et al. (2022)
SeNPs	*Gerbera jamesonii*	Promoted antioxidant defense system activity and endogenous hormone alterations	Khai et al. (2022)
ZnONPs	*Linum uitatissimum* L	Increased the growth and yield of flax, improved the levels of photosynthetic pigments, free amino acids and carbohydrates of flax plants	Sadak et al. (2020)
ZnONPs	*Cucumis sativus*	Increased the content of starch on cucumber fruit	Zhao et al. (2014)
ZnONPs	*Zea mays* L	Improved the activities of antioxidant enzymes, and reduced lipid peroxidation in the maize cell membrane system due to drought	Sun et al. (2020a)

(continued)

Table 8.1 (continued)

Nanoparticles	Plant	Responses	References
ZnONPs	*Zea mays* L	Improved the plant growth, biomass, and photosynthetic machinery in maize under cobalt (Co) stress by reducing ROS and MDA	Salam et al. (2022)
CuONPs	*Lactuca sativa* L	Enhanced growth and promoted the dry weight, total phenol content and flavonoid content of lettuce	Pelegrino et al. (2021)
CuONPs	*Coriandrum sativum*	yielded more biomass in cilantro	Zuverza-Mena et al. (2015)
CuONPs	*Cucumis sativus*	Increased the accumulation of metabolites such as sugars, organic acids, amino acids and fatty acids	Zhao et al. (2017)
CuONPs	*Hordeum vulgare L*	Enhanced seed germination parameters, and seedling growth parameters (roots and shoots' lengths, fresh biomasses and dry biomasses)	Kadri et al. (2022)
MgONPs	*Macrotyloma uniforum*	Increased the above-ground length, fresh biomass and the content of carbohydrate and protein	Sharma et al. (2021a)
SiO_2NPs	*Zea mays* L *Phaseolus vulgaris* L *Hyssopus officinalis* L. *Nigella sativa* L	Increased seed germination, root and shoot lengths, fresh weights (except for *Hyssopus officinalis* L.) and dry weights, photosynthetic pigments, total protein, and total amino acid (except for *Hyssopus officinalis* L.)	Sharifi-Rad et al. (2016)
SiO_2NPs	*Fragaria* × *ananassa* Duch	Treated with SiO_2NPs retained more photosynthetic pigments and exhibited higher levels of key permeates, such as carbohydrates and proline, and improved the drought tolerance by increasing the activity of antioxidant enzymes and reducing the degree of lipid peroxidation	Zahedi et al. (2020)

(continued)

Table 8.1 (continued)

Nanoparticles	Plant	Responses	References
SiO_2NPs	*Cucurbita pepo* L	Reduced cellular oxidative damage by enhancing antioxidant enzyme activity	Siddiqui et al. (2014)
SiO_2NPs	*Lavandula officinalis*	Enhanced the multiplication and growth of in vitro plantlets and modified its phytochemical compositions and essential oil bioactivities	Khattab et al. (2022)
MSNs	*Triticum aestivum* *Lupinus angustifolius*	Enhanced the germination of seeds and increased the content of plant biomass, total protein and chlorophyll	Sun et al. (2016)

which may be related to the reduction of hydrogen peroxide (H_2O_2), reactive oxygen species (ROS) and lipid peroxidation levels and the increase in the activities of glutathione reductase (GR), ascorbate peroxidase (APX) and CAT. Additionally, the increased activities of antioxidant enzymes and reduced the levels of ROS and malondialdehyde (MDA) by the application of AgNPs advocate stress ameliorative role against Cadmium (Cd) stress in carrot (*Daucus carota* L.) plant (Faiz et al. 2022). However, research on wheat (*Triticum aestivum* L.) found that AgNPs treatment increased the lipid peroxidation of wheat seedling tissues, indicating that exposure to AgNPs may have negative effects and toxicity problems on plants (Dimkpa et al. 2013; Siddiqi and Husen 2021).

8.2.2 Selenium Nanoparticles

Selenium (Se) is an essential micronutrient that promotes human and animal health, and it is also a beneficial element for plant growth (Hu et al. 2018). Early studies have demonstrated that low-dose sodium selenite spraying on foliage increased the Se content of grapes and winter wheat, and increased their growth and yield (Ducsay et al. 2006; Zhu et al. 2017). Se nanoparticles (SeNPs) have gained extensive interest due to their unique biological properties and have been recommended as a more effective, safer platform for Se delivery (Xu et al. 2018). Zahedi and colleagues (2019) assessed the potential effects of SeNPs in alleviating the adverse impact of soil salinity on strawberry (*Fragaria* × *ananassa* Duch.) plant growth and yield. The results showed that strawberry plants treated with SeNPs had higher contents of major osmolytes, such as free proline and total soluble carbohydrates, compared to the control group under stress conditions. Foliar spraying of SeNPs enhanced salinity tolerance in strawberries by decreasing soil-salinity stress-triggered H_2O_2

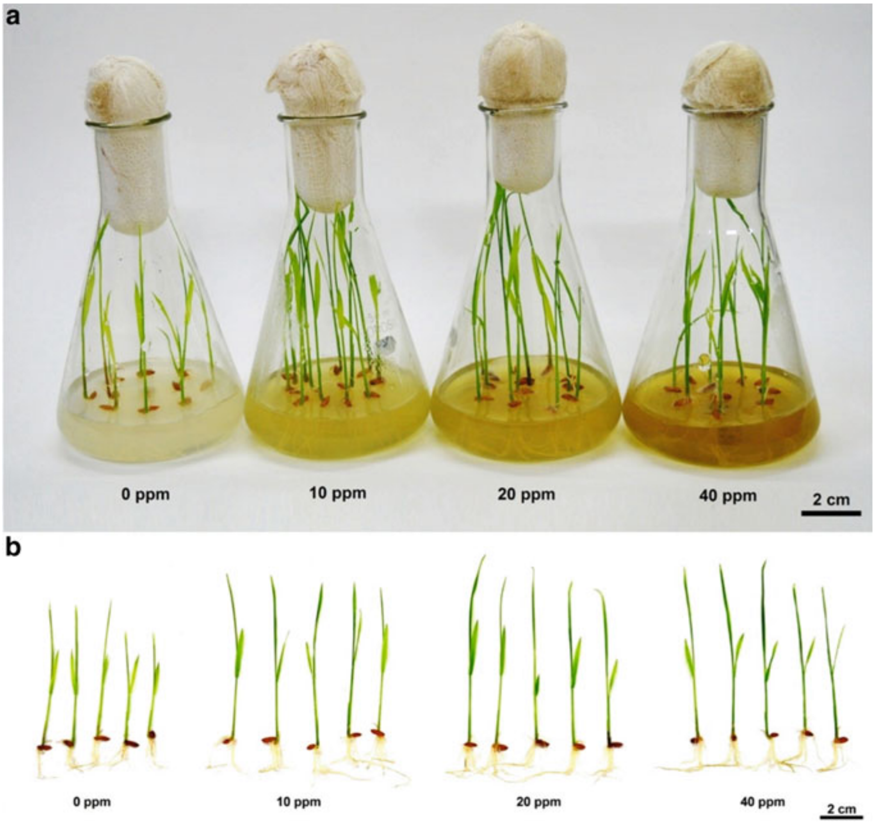

Fig. 8.2 Germination and seedling growth of rice on medium containing various concentrations of AgNPs at 14 d of incubation (**a**), and shoot and root elongation at 14 d under the exposure of AgNPs (**b**). *Source* Gupta et al. (2018)

level and lipid peroxidation via increasing superoxide dismutase (SOD) and CAT activities. In addition, spraying SeNPs (20 mg/L) on pepper (*Capsicum annuum* L.) leaves can increase the levels of chlorophyll and soluble sugars, thereby activating the branched-chain fatty acid and phenylpropane pathways, as well as the expression of AT3-related genes and enzymes. SeNPs treatment remarkably increased the levels of proline pathway-associated compounds, which can reduce the levels of malondialdehyde and hydroxyl free radicals in crops (Li et al. 2020). Studies have also shown that foliar application of SeNPs can reduce the lipid peroxidation and H_2O_2 content induced by drought stress by improving the activity and content of antioxidant enzymes, thereby reducing the harmful effects of oxidative stress on pomegranate (*Punica granatum* L.) fruits and leaves (Zahedi et al. 2021). It is also reported that SeNPs can reduce the oxidative stress and membrane lipid damage caused by Cd by inhibiting the expression of NADPH oxidase and glycolate oxidase in *Brassica napus* L. (Qi et al. 2021). In addition, Sardar et al. (2022) found that SeNPs promoted the alleviation of Cd toxicity via enriching leaf relative water content, total soluble sugars and chlorophyll content, improving gas-exchange parameters and modulating the antioxidant system of coriander (*Coriandrum sativum* L.) plants in response to Cd stress.

Due to the invasion of various pathogens including fungi and bacterial strains, food security and yield are seriously threatened (Bramhanwade et al. 2015). Among various nanoparticles, SeNPs have become powerful antibacterial agents and bio-enhancers to reduce the catastrophic effects of plant pathogenic species. Hashem et al. (2021) demonstrated that biogenic SeNPs improved *Vicia faba* (Faba Bean) morphological and metabolic determinants, yield and seed germination. Moreover, SeNPs have promising antifungal activity against *Rhizoctonia solani*, which can markedly enhance morphological and metabolic determinants as well as growth and yield compared to the infected controls. In summary, SeNPs have important potential applications in reducing biotic and abiotic stresses of plants (Zohra et al. 2021).

8.2.3 Zinc Oxide Nanoparticles

Zinc (Zn) is an important micronutrient necessary for adequate plant growth. It is a vital element for the production of chlorophyll, protein synthesis and carbohydrate metabolism, and is the key to biomass production (Anita 2021). Many reports have demonstrated that zinc-oxide nanoparticles (ZnONPs) may improve plant productivity and growth. Therefore, research related to the application of ZnONPs is of great significance to sustainable agriculture (Rizwan et al. 2017). ZnONPs can be used as nano-fertilizers to reduce the amount of fertilizer used without affecting crop yields and maximize crop productivity (Palacio-Márquez et al. 2021). Sadak et al. (2020) found that ZnONPs have beneficial effects on the growth and yield of flax (*Linum uitatissimum* L.) plants, which are manifested in improving the levels of carbohydrates, free amino acids and photosynthetic pigments of flax plants. Zhao and co-workers (2014) showed that ZnONPs (800 mg/kg of soil) could alter the carbohydrate quality of cucumbers (*Cucumis sativus*). The results indicated that ZnONPs might not influence the content of reducing and non-reducing sugars, but increased the content of starch on cucumber fruit. Sun et al. (2020a) found that ZnONPs (100 mg/L) significantly improved the activities of SOD, CAT and APX, and reduced lipid peroxidation in the maize (*Zea mays* L.) cell membrane system due to drought. In addition, ZnONPs enhance the biosynthesis of starch and sucrose in maize under drought stress by elevating the levels of UDP-glucose pyrophosphorylase (17.8%), glucose phosphate isomerase (391.5%) and cytoplasmic invertase (126%) (Sun et al. 2020b). Recently, Salam et al. (2022) demonstrated that ZnONPs improved the plant growth, biomass, and photosynthetic machinery in maize (*Zea mays* L.) under cobalt (Co) stress by reducing ROS and MDA. However, the accumulation of a high dosage of NPs in plants can affect their growth. Studies have confirmed that a high dosage of ZnONPs causes oxidative stress in wheat (*Triticum aestivum*) by increasing the level of lipid peroxidation and oxidizing glutathione in roots (Dimkpa et al. 2012). Salehi et al. (2022) found that aerially applied ZnONPs negatively affect fully developed bean plants (*Phaseolus vulgaris* L.). ZnONPs could induce tapetum abnormality, abnormal deposition of carbohydrates, and eventually apoptosis.

8.2.4 Copper Oxide Nanoparticles

Copper (Cu) is an essential micronutrient for plants, directly involved in the synthesis of oxidoreductase and the metabolism of protein and carbohydrates (Wang et al. 2019). Due to the antibacterial properties of copper oxide nanoparticles (CuONPs), they are being used commercially for wood preservation, agricultural fungicides and antifouling coatings (Yuan et al. 2016). The application of CuONPs in agriculture is relatively new, but interest in its use as plant protection products and nano-fertilizers is increasing (Verma et al. 2018). CuONPs can be used as nutrients for plants but can have phytotoxicity depending on its application concentration, particle size, administration method and exposure time (Liu et al. 2016). There are facts that at low concentrations CuONPs have beneficial effects on plants. Pelegrino et al. (2021) found that spraying spherical CuONPs on lettuce (*Lactuca sativa* L.) leaves had a positive effect on its growth and promoted the dry weight, total phenol content and flavonoid content of lettuce. The CuONPs synthesized by green tea at concentrations between 0.2 and 20 μg/mL are non-phytotoxic and might enhance lettuce radicle growth (Pelegrino et al. 2020). Zuverza-Mena and co-workers (2015) found that compared with the control, application of CuONPs improved the biomass yield of cilantro (*Coriandrum sativum*). Zhao et al. (2017) found that compared with the control, administration of CuONPs significantly increased the accumulation of metabolites (e.g., sugars, fatty acids, amino acids and organic acids) in cucumbers (*Cucumis sativus*). Recently, Kadri et al. (2022) reported that enhancement of *Hordeum vulgare* L. seed germination parameters, and seedling growth parameters (shoots and roots' lengths, dry biomass and fresh biomass) by decreasing the concentration of CuONPs. However, at present, most researches have focused on the toxicity of CuONPs to crops. Exposure to CuONPs can cause membrane lipid damage, increased ROS and proline accumulation, and decreased seed germination in rice (*Oryza sativa* L.) seedlings (Shaw et al. 2013). Nair et al. (2015) reported that CuONPs reduced the shoot elongation, carotenoids and chlorophyll content of *Indian mustard*. Therefore, in order to take advantage of the benefits of CuONPs on plant growth and development as well as disease control of various crops, further research especially optimal dosage is needed.

8.2.5 Magnesium Oxide Nanoparticles

Magnesium (Mg) is the fourth most important element after potassium (K), phosphorus (P), and nitrogen (N) among the important elements for plant development.

It participates in many physiological and biochemical reactions during plant growth and development (Cakmak 2013). Mg is essential for the formation of plant carbohydrates and protein. 75% of the Mg in plant leaves is used for the synthesis of plant protein, 20% is involved in the synthesis of chlorophyll, and is used as an enzyme cofactor to participate in the carbon fixation and metabolism process of photosynthesis (Cakmak et al. 2008). Mg deficiency can inhibit the synthesis of chlorophyll, cause different morphological and physiological changes in plants, and regulate its secondary metabolic pathways (Guo et al. 2016). Magnesium oxide nanoparticles (MgONPs) are easily absorbed by the soil, improve fertilizer utilization, and can be used as an element supplement to increase the Mg content in the soil. When *Macrotyloma uniforum* was treated with MgONPs, the above-ground length, chlorophyll content and fresh biomass increased significantly, and the accumulation of carbohydrates and proteins increased by 4–20% and 18–127% (Sharma et al. 2021a). However, some studies have found that exposure to MgONPs negatively regulates plant growth. Sharma et al. (2021b) found that treatment with MgONPs resulted in reductions in total chlorophyll, biomass and carbohydrate content of mungbean (*Vigna radiata* L.) by 24–75%, 40% and 41%, respectively. These studies indicated that responses to MgONPs are largely dependent on the type of plants.

8.2.6 Silicon Dioxide Nanoparticles

Silicon (Si), the second most abundant element on Earth's crust, is abundantly found in the soils. Si can facilitate plants to cope with abiotic and biotic stresses (de Moraes et al. 2021). Sharifi-Rad co-workers (2016) studied the effects of silicon dioxide nanoparticles (SiO_2NPs) on two field crops (*Phaseolus vulgaris* L. and *Zea mays* L.) and two medicinal plants (*Nigella sativa* L. and *Hyssopus officinalis* L.) on the morphological and biochemical characteristics. The results showed that 400 mg/L SiO_2NPs remarkably improved shoot and root lengths, dry weight and fresh weight (except for *Hyssopus officinalis* L.), photosynthetic pigments, seed germination, total amino acid (except for *Hyssopus officinalis* L.) and total protein, but total carbohydrates appeared to reduce. Zahedi et al. (2020) found that spraying a solution containing SiO_2NPs improved the growth of strawberry (*Fragaria* × *ananassa* Duch.) plants under normal and drought stress conditions. Compared to the control group, the plants treated with SiO_2NPs retained more photosynthetic pigments and exhibited higher levels of key permeates, such as carbohydrates and proline. In addition, exogenous spraying of SiO_2NPs improves the drought tolerance by elevating the activities of antioxidant enzymes and reducing the degree of lipid peroxidation. Siddiqui et al. (2014) reported that SiO_2NPs can significantly reduce cellular oxidative damage by enhancing antioxidant enzyme activity, reducing MDA and H_2O_2 levels, and alleviating the decrease in pumpkin (*Cucurbita pepo* L.) seed germination rate, vitality, and growth caused by salt stress. Recently, Khattab et al. (2022) found that SiO_2NPs enhanced the growth and multiplication of *Lavandula officinalis* in vitro plantlets and modified its essential oil bioactivities and phytochemical compositions.

Mesoporous silica nanoparticles (MSNs), as a kind of SiO_2NPs, are widely used in various fields of agriculture because of their large specific surface area, uniform particle size, high stability, easy to modify internal and external surfaces and good biocompatibility (Popat et al. 2012; Abdelrahman et al. 2021). Sun and co-workers (2016) found that the photosynthesis efficiency of wheat (*Triticum aestivum*) and lupin (*Lupinus angustifolius*) plants exposed to MSNs was significantly improved, the germination of seeds was enhanced, and the plant biomass, total protein and chlorophyll content increased.

8.3 Green Synthesis of Nanoparticles Using Plant Carbohydrates

NPs have been prepared by various approaches, including biological, chemical and physical methods. Compared to chemical and physical methods, biological entities, especially plants, provide an environment-friendly, safe and efficient method for the synthesis of NPs (Nayantara et al. 2018). Recently, plant extracts (phenolic acids, bioactive alkaloids, polyphenols, terpenoids, sugars and proteins) have been demonstrated to serve as reducing, stabilizing, and/or complexing agents for the green synthesis of NPs (Ettadili et al. 2022). Carbohydrates with different functional groups (e.g., amino, carboxylate, ester, hydroxyl and sulfate) can bind with metal precursors via non-covalent or van der Waals interaction, leading to the decreased levels of metals and stabilization of metal NPs (Majhi et al. 2021). As the main carbohydrate, polysaccharides contain various functional groups, including hydroxyl groups capable of reducing precursor salts and hemiacetal reducing ends (Fig. 8.3). The carbonyl groups oxidized from polysaccharide hydroxyl groups play a vital role in the reduction process of inorganic salts. The reducing ends of polysaccharides have been applied to introduce amino functional groups that can compound and stabilize NPs (Park et al. 2011).

The synthesis of NPs from carbohydrates has attracted more and more attention because of its non-toxic, safe, stable, good biocompatibility, environmental friendliness and strong availability of carbohydrates (Boddohi et al. 2010). The use of natural polysaccharides as reducing agents and stabilizers for NPs synthesis is a simple green synthesis technology, which does not require any other chemical reducing agents (Mohammadlou et al. 2016). Kumar et al. (2019) used *Arabidopsis thaliana* as a model plant for the biosynthesis of AgNPs *in vitro* and systematically revealed the biochemical components required for the production of AgNPs. The results showed that carbohydrates, polyphenols and glycoproteins are the basic factors for stimulating the synthesis of AgNPs. Yugay et al. (2020) isolated polysaccharides (alginate, fucoidan, and laminaran) from the seaweed *Fucus evanescens* and *Saccharina cichorioides*, and found that all polysaccharides can be used as reducing agents to convert silver nitrate into AgNPs, and their catalytic activities may vary as follows: laminaran > fucoidan > mayalginate. Tippayawat et al. (2016) used aloe

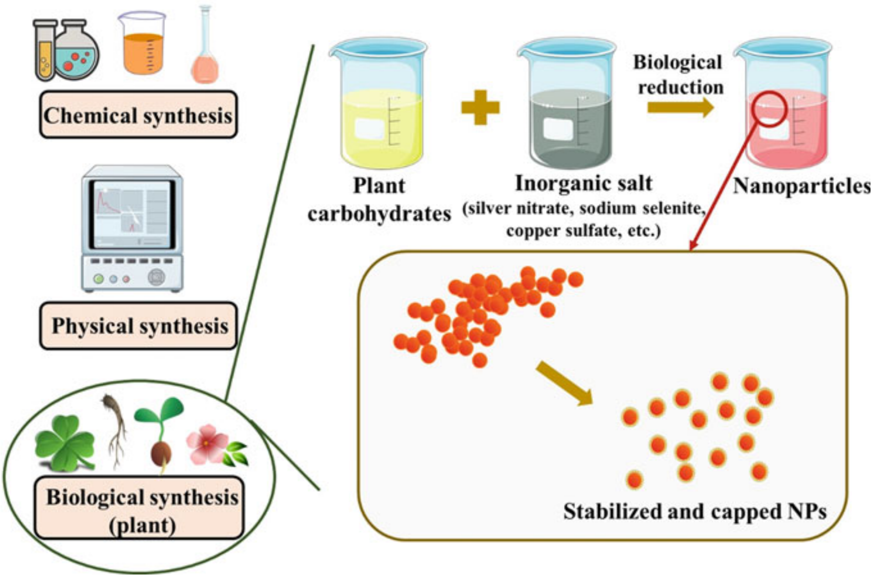

Fig. 8.3 Green synthesis of nanoparticles using plant carbohydrates. Graph by Lei Qiao

plant extracts rich in pectin, lignin and hemicellulose to synthesize spherical AgNPs, in which the aloe vera extract functions as both capping and reducing agents. Zhou et al. (2021) synthesized stable and individual spherical SeNPs with a mean diameter of ~ 79 nm using the *Citrus limon* L.-extracted polysaccharides as the decorator and stabilizers and SeNPs exhibited good dispersion and high stability in water for at least 3 months. Jonn et al. (2017) reported an environmentally friendly synthesis of platinum nanoparticles (PtNPs) using water hyacinth (*Eichhornia crassipes*) plant extracts as effective reducing agents and stabilizers. Fourier transform infrared spectroscopy (FTIR) showed that the extracts hydroxyl, nitrogen and carbohydrate groups are responsible for the reduction and capping of PtNPs. Patil and co-workers (2016) developed cerium oxide (CeO_2) NPs with a mean particle size of < 40 nm using a pectin extracted from the peel of red pomelo (*Citrus maxima*). In general, the synthesis of nanoparticles using carbohydrates has more advantages than traditional approaches in terms of cost, eco-friendly and biocompatibility.

8.4 Conclusion and Prospects

NPs have become a research hotspot in the field of agriculture due to their special physical and chemical properties. This article mainly reviews the application of NPs in sustainable agriculture, such as promoting plant growth and improving stress resistance, from the perspective of plant macromolecules (carbohydrates and lipids).

In addition, the use of plant macromolecules to biosynthesize nanoparticles is cost-effective, environmentally friendly and can improve the performance of NPs. Many researchers have confirmed that biosynthetic NPs are an effective strategy to replace traditional nanoparticles and agrochemicals. Therefore, the biosynthesis of NPs can inevitably become an emerging trend in the sustainable development of agriculture. Natural products, such as plant carbohydrates, can serve as promising candidate materials for the green synthesis of NPs, which are abundantly available in nature. While developing nanotechnology in the future, the safety of NPs, especially their toxicity, should be considered, and their safe use range and use environment should be studied to improve their utilization and stability, and promote the application of NPs in sustainable agriculture.

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Chapter 9
Interaction of Nanomaterials with Plant Macromolecules: Nucleic Acid, Proteins and Hormones

Roseanne Mascarenhas, Tanvi Mathur, Jaya Maheshwari, and Praveen Nagella

Abstract Nanotechnology has the ability to change a wide range of industrial and agricultural operations. To harness these possibilities, it is essential to construct nanomaterials that have minimum impact on the human body, plant systems as well as the environment. Using different materials can up- or down-regulate diverse genes of plants, create stimulating or stressful conditions and even cause production of metabolites that affect plant-associated microbes. The same nanoparticle can promote one plant species' growth and be toxic to another. A small change in the concentrations could cause either flourishment or senescence. It is crucial to understand how nanomaterials interact with nucleic acids, the most fundamental plant macromolecule, as well as with the proteins and hormones made by biochemical processes. This chapter explores the basics of nanotechnology, with a brief classification and notes on some of the most recently used nanomaterials in agriculture such as metals and their oxides, quantum dots, graphene, arabinoxylan and chitosan nanoparticles, single and multi-walled carbon nanotubes. Interactions with these above-mentioned macromolecules are explored, along with futuristic applications in plants that are currently being tested, like nanocarriers and nanovalves. Through this work, it is hoped that the field will further be extended through proper understanding of the environmental implications of nanomaterials, and that green technology will become the norm.

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9.1 Introduction

Materials with at least one dimension less than 100 nm are referred to as nanomaterials. The study of different nanoparticles (NPs) with sizes ranging from 1 to 100 nm, at least in one dimension, is therefore referred to as nanotechnology (Yang et al. 2020). Nanotechnology is the practice of producing micro and macro materials and goods with atomic precision. The US National Science and Technology Council's (NSTC 2000) shortest and most frequently cited definition of nanotechnology is as follows: "The essence of nanotechnology is the ability to work at the molecular level, atom by atom, to create large structures with fundamentally new molecular organization. The aim is to exploit these properties by gaining control of structures and devices at atomic, molecular, and supramolecular levels and to learn to efficiently manufacture and use these devices."

According to the official definition of the United States National Science Foundation (Roco et al. 1999), investigations involving materials and systems with the following crucial characteristics fall within the category of nanoscience/nanotechnology:

(a) At least one dimension between 1 and 100 nm counts as a dimension.
(b) The design process's methodologies show that the physical and chemical characteristics of molecule-sized objects can be fundamentally controlled.
(c) The ability to be joined to create larger constructions or a building block property (Ghorbanpour et al. 2017).

The importance of nanotechnology has become greater for a variety of specific reasons, including:

(a) The quantum mechanical (wavelike) characteristics of electrons inside matter are affected by variations on the nanoscale. It is possible to modify the macroscopic and microscopic characteristics of materials, such as charge capacity, magnetism, and melting temperature, by designing them at the nanoscale.
(b) The regular arrangement of matter at the nanoscale is a crucial characteristic of biological beings. We can now insert manufactured tiny objects within living cells thanks to advancements in nanoscience and nanotechnology. Using molecular self-assembly, it is also possible to analyze the micro- and macrostructure of materials. This is unquestionably a useful tool for material science.
(c) Due to their extremely high surface-to-volume ratio, nanoscale components are perfect for application in energy storage, composite materials, reacting systems and drug delivery.
(d) The density of macroscopic systems composed of nanostructures can be substantially higher than that of systems composed of microstructures. They might also function as superior electrical conductors. This could lead to the development of

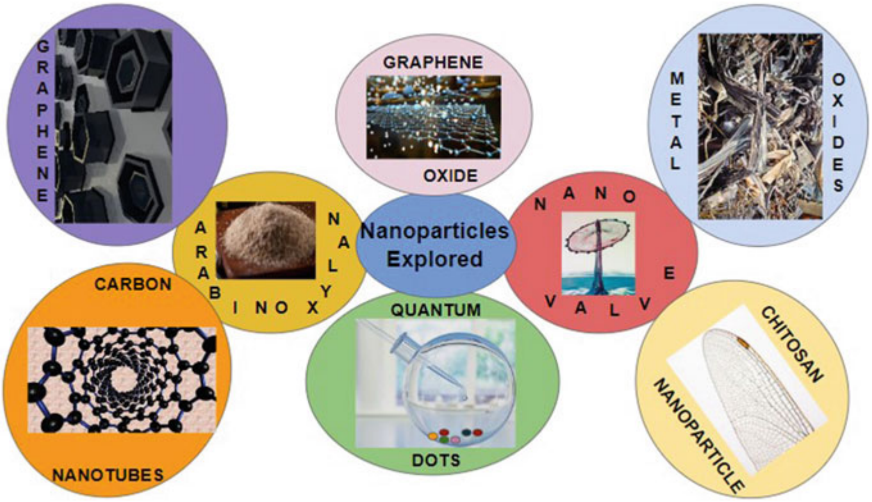

Fig. 9.1 Some of the major nanoparticles (Elements in this image have been created with the help of pixabay. https://pixabay.com/)

smaller, quicker electronic device concepts, circuits, more complex features, and significantly less power consumption concurrently by regulating the complexity and interactions of nanostructures (Ghorbanpour et al. 2017).

A wide variety of NPs, including metal NPs, quantum dots, mesoporous silica NPs, clay nanosheets, DNA nanostructures, and carbon nanomaterials including graphene, carbon nanotubes and carbon dots (Fig. 9.1), have been employed in plant biotechnology (Sanzari et al. 2019). The objective of this chapter is to explore the properties of nanomaterials; the differences in their synthesis and functionalization leading to altered interaction with plant life. It focuses on the effect on plant growth and on macromolecules like nucleic acids, proteins and hormones, elucidating the chemical interaction between key residues. This chapter will finally demonstrate how nanomaterials can be designed for highly sensitive functions within a living plant system to regulate the release of growth hormones. Through this it is hoped that future research will engineer a symbiosis between eco-friendly nanomaterials and plants designed to accommodate them as part of their biology.

9.2 Classification of Nanomaterials

The two primary categories of NPs are organic and inorganic. Micelles, dendrimers, liposomes, hybrid, and compact polymeric NPs are included in the first group. Fullerenes, quantum dots, silica, and metal NPs are included in the second group.

Based on their dimensions, NPs can be zero-dimensional (all dimensions are at the nanoscale), one-dimensional (this includes nanotubes, nanorods, and nanowires), two-dimensional (plate-like shapes, includes graphene, nanofilms, nanolayers, and nano-coatings), or three-dimensional (of any shape; this class can contain bulk powders, dispersions of NPs, bundles of nanowires, and nanotubes as well as multi-nanolayers) depending on how they interact with the material (Ray 2018). NPs are classified into three types based on their origin: natural, accidental, and artificial NPs (Kole et al. 2016).

Natural—Naturally-occurring NPs have been present ever since the creation of Earth. They are present in a variety of materials, including mineral composites, photochemical reactions, forest fires, simple erosion, lunar dust, terrestrial dust storms, and volcanic dust.

Incidental—The majority of incidental NPs are produced by industrial processes that humans have created, such as exhaust from cars and trucks, coal combustion, welding gases, and industrial exhausts.

Artificial—Carbon-based (CB NPs), metal-based (MB NPs), magnetic (MB NPs), dendrimers, and composite NPs are the five different types of designed NPs. Examples of carbon-based NPs (NPs) include fullerene (C70), fullerol [$C_{60}(OH)_{20}$], single-walled carbon nanotubes (SWCNTs), multiwalled carbon nanotubes (MWCNTs), and single-walled carbon nanohorns (SWCNHs), whereas metal-based NPs are made of nanomaterials based on gold (Au), silver (Ag), copper (Cu), and iron (Fe).

Anthropogenic nanomaterials have 2 classes: carbon base and the metal base (Peralta-Videa et al. 2011). There are several morphologies, including spheres, cubes, rods, wires, plates, prisms, core–shell structures, and intricate 3D architectures, which change shape or agglomerate in response to external stimuli, modifying their chemical or physical properties (Keller et al. 2010). There are three main classes of nanobiosensors: metal/metalloid, quantum dots, and array-based (Li et al. 2020).

Some significant kinds of NPs are based on physical and chemical properties, including:

Carbon-based—(fullerenes, carbon nanotubes, graphene, carbon dots). These materials are particularly intriguing because of their electrical conductivity, high strength, structure, electron affinity, and flexibility.

Metal—They are entirely composed of the predecessors to metals. These have distinctive optoelectrical capabilities as a result of their well-known localized surface plasmon resonance (LSPR) features.

Ceramics—Due to their usage in processes like photocatalysis, photodegradation of dyes, imaging, and catalysis, these inorganic nonmetallic solids are attracting a lot of study interest.

Semiconductor—Semiconductor materials have broad band gaps and characteristics that fall between those of metals and non-metals. Their properties are significantly altered as a result of band gap tuning. As a result, they play a crucial role in electrical, photocatalytic, and photo- optical systems.

Polymers: For the synthesis of polymeric NPs for a variety of uses, including surface coating, sensor technology, catalysis, and nanomedicine, scientists have devised a number of approaches.

Lipids—These NPs are used as drug carriers in numerous biomedical applications because they include lipid moieties (the mRNA Covid-19 vaccines are using lipid nanotechnology). Another extremely promising method for delivering nucleic acids in gene therapy is lipid NPs (Ray 2018).

9.3 Major Types of Nanoparticles

9.3.1 Metal Oxide NPs

Metal oxide NPs release bivalent ions which could be the basis for interaction and upregulation of genes (Fig. 9.1). Amorphous, spherical, dispersed silica NPs form colloidal SiNPs. They exhibit aggregation in solution as indicated by their high polydispersity index (PDI) of 5.11. Zeta (ζ) potential is 11.2 mV and their size is 24 nm, indicating that they are stable with a negative surface charge at physiological pH as a result of the ionization of the hydroxy groups (–OH). The functionalized SiNPs containing amino acids and carbohydrates is revealed by X-ray diffraction (XRD) to have an amorphous structure like that of pure SiNPs. Through electrostatic interactions or hydrogen bonds, the functional groups are incorporated into the silica's pore volume. Using covalent chemistry, physical adsorption, or electrostatic interactions, surface modification is possible. For surface modification by replacing previously attached functional groups, non-covalent attachment is quick and inexpensive (Kumar et al. 2021). The effects of ZnO, CuO and CdS quantum dots are similar, possibly due to faster dissolution or innate physico-chemical characteristics (Pagano et al. 2018). CuO nanomaterials in the crystal phase were created by Mazaheri-Tirani and co-workers, which were monoclinic, spherical and black in colour, with a diameter of 50 nm, and surface area over 100 $m^2\ g^{-1}$. They have a true density of 6.4 g cm^{-3}, and an aerodynamic particle size of less than 30 nm. These could be delivered through hydroponics (Mazaheri-Tirani et al. 2021). In a study by Zhao et al. the size of 30 nm Fe_3O_4 particles created had the properties of being magnetic, catalytic, antimicrobial and adsorbent (Zhao et al. 2019). Other physical characteristics of NPs used in that study are described in Table 9.1.

Table 9.1 Physical characteristics of metal oxide NPs

NPs	Size (nm)	Hydrodynamic diameter (nm)	Zeta potential (mV)
Fe_3O_4	30	1230 ± 56	11.6 ± 0.64
TiO_2	5–10	592 ± 7	−21.4 ± 0.64
SiO_2	20	876 ± 41	19.5 ± 0.37

Notably, multiple investigations showed that charged NPs can be directed to specific tissues within plants for delivery and formation of aggregates there. MgONPs in the extraction solution have a negative charge. These interactions could therefore be a vitally improved mechanism for accelerating absorption. Magnesium is a crucial mineral, and MgONPs may one day be employed to treat magnesium deficiencies in plants used in agriculture. MgONPs might ameliorate the stress of magnesium deficiency on the control plant cell membrane, as seen by the anomalous decrease in the MDA concentration (Cai et al. 2018).

9.3.1.1 Quantum Dots

Quantum dots (QDs) are stable colloidal nanocrystals with a semiconductor core of cadmium sulfide, selenide or telluride, and an outer shell that prevents oxidation and leaching of the heavy metal. Its optical properties improve photoluminescence and quantum yield (Mo et al. 2017). Disposed QDs from electronic devices accumulate in landfills at ng/kg to µg/kg levels, causing Cd^{2+} toxicity (Majumdar et al. 2019a, b).

9.3.1.2 Graphene

With a single-layer thickness of 0.8–1 nm, graphene is “the king of novel materials” because it is the lightest, strongest, and most thermally and electrically conductive novel artificial nanomaterial. A single-layer honeycomb lattice of cross-linked benzene rings makes up the two-dimensional structure. Multi-layer graphene, graphene oxide (GO), and reduced GO are some other forms. For greater solubility and biocompatibility, a gradient of hydrophilicity to hydrophobicity exists from the edge to the centre of the graphene sheet (Yang et al. 2020).

9.3.1.3 Arabinoxylan

The wheat industry produces 25% bran as a byproduct, which is a rich source of the hemicellulosic water-soluble polysaccharide arabinoxylan (AX). It is highly branched and made of two pentose sugar units: β−(1−4)-xylan backbone branched with a very short chain α−(1−2) and α− (1−3) linked arabinose. This gives it a self-assembling property through multivalent topology, hydrophobic interactions, Van der Waal’s forces and hydrogen bonding. The many hydroxyl (–OH) groups along its backbone can undergo esterification and etherification, forming local interaction sites for self-assembly. This can transfer unstable nucleic acids and other small or large-molecule, bioactive chemicals to plants (Berlanga-Reyes et al. 2009). These NPs can be employed in green technology for non-immunogenic formulation excipients and as effective transfection agents, particularly for CRISPR-Cas9 DNA

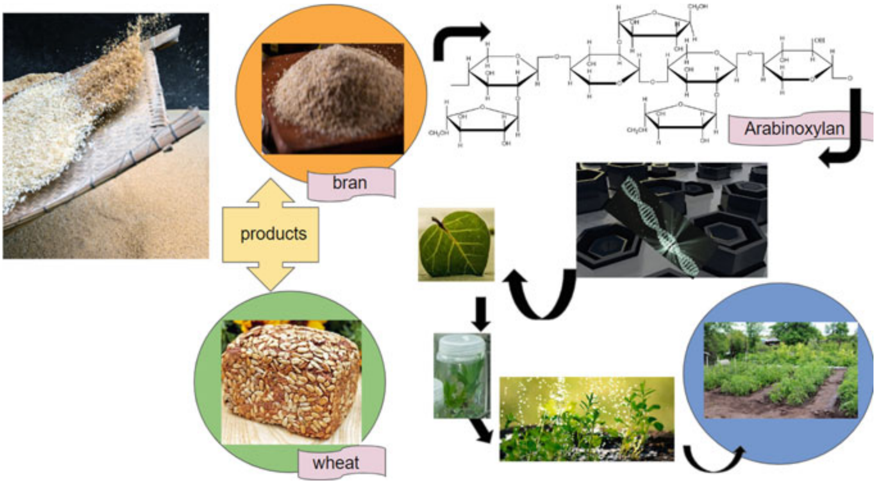

Fig. 9.2 Arabinoxylan NPs- production and use (Elements in this image have been created with the help of Pixabay. https://pixabay.com/)

constructs, because they are nontoxic and biodegradable. Bran can thus be a product with added value.

Van der Waals interactions are facilitated by local inclusion of charges along the backbone enabling spontaneous and thermodynamically driven self-assembly. Succinic anhydride and dimethylformamide (DMF) were used to make anionic carboxylic acid terminated AX, which replaces –OH groups. For cationic AX, glycidyl trimethylammonium chloride (GTMAC) and sodium hydroxide were used to transfer quaternary nitrogen (Sarker et al. 2020) (Fig. 9.2).

9.3.1.4 Single Walled Carbon Nanotubes (SWNT)

Nucleic acid delivery and biosensing are two applications for single walled carbon nanotubes (SWNT). *Agapanthus praecox* (F. M. Leight) embryogenic calli's cryopreservation is improved by SWNTs' reduction of oxidative stress (Ren et al. 2020). Their toxicity is concentration dependent in protoplasts of *Arabidopsis thaliana* Willdenow and rice. Chemical functionalization of SWNTs also affects toxicity (Shen et al. 2010).

9.3.1.5 Chitosan

Chitosan produces diverse disease resistance in plants. It is an elicitor molecule for many host–pathogen interaction studies, with biodegradability, non-toxicity, and antibacterial properties. Chitosan nanoparticles (CNP) are created by cross linking tripolyphosphate with the ammonium groups in CNP (Qi et al. 2004) in inter- and intramolecular interactions. CNP is a versatile anti-pathogenic or phytosanitary chemical for more extensive sustainable organic cultivation.

9.3.2 Nanocarriers and Nanovalves

Both organic and inorganic nano compounds made out of silica, metals and their oxides, carbon structures, micelles from polymers, and dendrimers, are used for the delivery of agrochemicals. Their low cargo capacity along with uncontrollable release and lack of biodegradability prevents their widespread use in agriculture. Hybrids called metal–organic frameworks (MOFs) are more recently being used for catalysis, optics, sensing and detection, as they are easy to synthesize and accessorize through functional groups. They have diverse composition and flexible structure, with adjustable pore size, chemical or colloid stability and improved cargo capacity. They are more biocompatible and biodegradable. Amongst them, supramolecular macro cycles are stimuli-responsive. These have application in the delivery of plant hormones only under a particular condition such as stress (Yang et al. 2021).

9.4 Role of Nucleic Acids, Proteins and Hormones in Plants

Large molecules known as nucleic acids carry all of the genetic information and a tonne of tiny details. Every living entity that consumes and transforms energy, including bacteria, viruses, fungi, plants, and mammals, has nucleic acids. Deoxyribonucleic acid, or DNA, and ribonucleic acid (RNA) are the two different forms of nucleic acids. Nucleic acids are composed of strands of nucleotides, which are composed of a phosphoric acid, a sugar with five carbon atoms, and a nitrogenous base. A gene is made up of certain nitrogenous base clusters along a DNA strand. A gene is a structure that carries hereditary information to the following generation and contains the genetic information or product codes. However, genes are not restricted to reproductive cells. Because DNA also includes the protein-coding information for the organism's proteins, every cell in an organism possesses DNA (and thus genes).

Additionally, proteins give cells structure and control biological activity. Most biological processes are carried out by proteins in cells. Thus, proteins can be viewed as biomolecular machines with unique structural and functional properties that are frequently challenging to imitate in a laboratory setting. Different enzymatic, structural, and functional roles are performed by plant proteins (photosynthesis, biosynthesis, transport, immunity). They act as storage containers in addition to meeting the nutritional and growth needs of developing seedlings. Proteins play these roles based on their make-up and distinctive structural forms, such as folding, which can range from compact and well-ordered to unfolded and essentially disordered. RuBisCo, albumins, globulins, prolamins, glutelins, and gliadins, are a few examples of the most crucial proteins found in plants (Rasheed et al. 2020).

For growth and development, plants require the external elements of nutrients, water, sunlight and oxygen. Several inherent elements also control their growth and development. These are referred to as "Phytohormones," or plant hormones. They are crucial in processes like vernalization, phototropism, seed germination and dormancy. Chemical substances known as plant hormones are found in extremely small amounts in plants. They are adenine (Cytokinins), indole (Auxins), terpenes (Gibberellins), carotenoids (Abscisic Acid), and gas derivatives (Ethylene). Almost every portion of the plant produces these hormones, which are then sent to various areas of the plant. They might work together or separately, playing either complementary or antagonistic roles. Given their importance, exogenous applications of synthetic plant hormones are used to regulate crop yield. Hormones are classified based on whether they promote or inhibit plant growth. Auxins are located in the growing apices of roots and stems before migrating to other areas to perform their functions. Indole-3-acetic acid (IAA) and indole butyric acid (IBA) are naturally occurring; 2,4-D (2,4-Dichlorophenoxyacetic acid) and Naphthalene acetic acid (NAA) are synthetic. Their functions include stem and root cell elongation, apical dominance and parthenocarpy to prevent early senescence, and for beginning the rooting process during grafting. It also promotes flowering, for example, in pineapple. The auxin 2,4-D is a common herbicide used to eradicate unwanted dicot weeds without harming monocot plants.

There are over 100 known gibberellins (GA_1, GA_2, GA_3, etc.) possessing an acidic character. They promote bolting in rosette plants like cabbage and beets, cause stem elongation and dwarfism reversal, parthenocarpy and postpone senescence. They cause starch mobilization within the endosperm through the production of hydrolytic enzymes like lipase and amylase, and they awaken dormant seeds. The process of cytokinesis depends on cytokinins. They are made in early fruits, shoot buds, and root apices, among other places. The migration of cytokinins is polar and basipetal. They encourage shoot growth, development of leaf chloroplasts, combat apical dominance, promote nutrient mobilization and prevent leaf withering. Natural cytokinins include zeatin (coconut milk and corn kernels) and isopentenyladenine. Synthetic ones are thidiazuron, kinetin, benzyladenine, and diphenylurea. Abscisic acid is a hormone that inhibits growth. GAs are counteracted by ABAs. It controls dormancy and abscission while inhibiting plant metabolism. Because it raises plants' stress tolerance, it is also known as "stress hormone". Ethylene is both an inhibitor and a growth promoter. It is a gas created in tissues going through senescence and in ripening fruits. It breaks seed and bud dormancy, controls leaf epinasty, hastens fruit ripening, and stimulates the rapid elongation of petioles and internodes. It is one of the most commonly utilized hormones in agriculture (Gaspar et al. 1996).

9.5 Importance of Interaction Between Nanomaterials and Macromolecules

Nanoparticulate structures can traverse plant cell walls and can be fine-tuned in size, shape, physical, mechanical and optical properties for diverse cargo conjugation (Cunningham et al. 2018). To be used in agriculture, they must have biocompatible source materials and nontoxic synthetic pathways. “Green nanotechnology” is coming into place, where plant- and biobased materials can be used to synthesize nanoscale systems with new form and function.

Different NPs interact with diverse plants in unique ways. Nanomaterials with higher surface activity can decrease energy flow between interfaces (Yang et al. 2020). Nanomaterial fullerene aids in the response of *Zea mays* L. cells to oxidative stress caused by cobalt (Co). It cancels out the effects on stomatal control, gas exchange, and water parameters of content and osmotic potential. It safeguards the PSI-PSII photosystems and photosynthetic machinery (Ozfidan-Konakci et al. 2022). Maize grown in soil treated with 100 mg/kg of SiO_2, TiO_2, or Fe_3O_4NPs for four weeks showed significant changes in the carbon and nitrogen profiles of the leaves and roots, as well as in metabolomics. They cause the plant roots to release metabolites like organic acid and nicotianamin that chelate Cu ions in the soil and lowered its bioavailability (Zhao et al. 2019). Nano-CuO of concentrations 0.1 and 2.5 μM increase shoot fresh weight of *Ruta graveolens* L., but only 2.5 μM increased the dry weight while decreasing root water content. 50 mg/l led to decreased shoot length, and high concentrations caused stomata closure, slowing growth, increased photosynthetic energy loss during the violaxanthin cycle, and decrease in total chlorophyll (Mazaheri-Tirani et al. 2021). The effects of MgONPs exposure on enhanced Mg absorption, growth stimulation, and several favorable morpho-physiological changes was seen in tobacco plants. After 30 days of treatment, MgONPs significantly enhanced the chlorophyll a and b contents from 0.21 and 0.12 g/g to 1.21 and 0.67 g/g respectively (Cai et al. 2018). Jhansi et al. also reported that plant leaves’ chlorophyll content can be increased by soaking peanut seeds in 500 mg/mL slurry of MgONP (particle sizes varying from 10 to 80 nm) for 12 h (Jhansi et al. 2017). Due of NPs’ high surface reactivity, which leads to increased hydromineral transportation in the roots, the roots can grow and produce root pores. Exposure to MgONPs may result in a significant increase in Mg concentration, which may enhance plant photosynthetic efficiency. MgONPs showed an advantage over other nanomaterials since plants subjected to 250 mg/mL of MgONP did not exhibit any significant damage or stress, indicating that the plants probably have enzymatic defences to handle relatively low MgONP concentrations. Its non-toxic effects are further supported by the cortex, epidermis, and pericycle of the MgONP-treated and untreated plants in the study, which all grew well with compact structures in the root, stem, and leaves. The plants exposed to MgONPs showed no adverse effects, such as distortion, curling, chlorosis, or necrosis in the leaves. NPs can widen existing root pores and enhance nutrient and hydromineral absorption because of their strong surface reactivity (Cai et al. 2018) (Fig. 9.3).

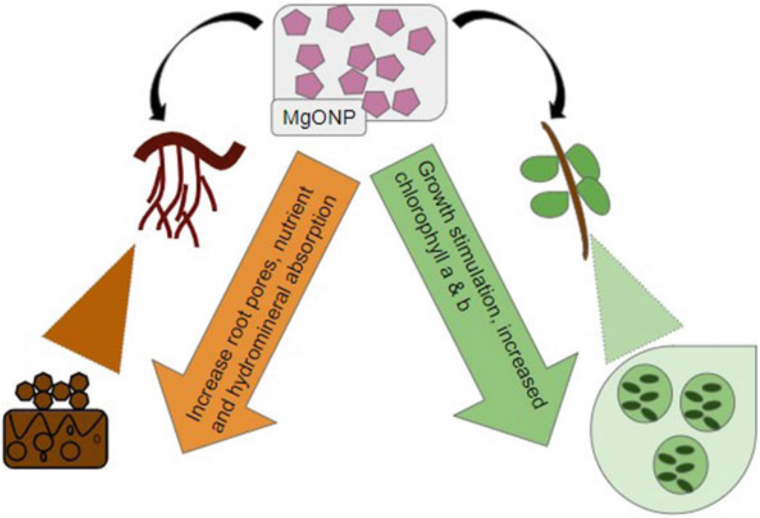

Fig. 9.3 Effect of MgONPs on root and shoot growth of *Nicotiana tabacum* L. (Elements in this image have been created with the help of Pixabay. https://pixabay.com/)

Iron NPs caused an increase in root length in *Arabidopsis thaliana* by cell wall loosening mediated by nZVI-mediated OH radical (Kim et al. 2014). Hydrolyzed collagen or sodium alginate has good strength of thermoplastic starch (TPS), which are biodegradable nanocomposites that induce protective effects for loquat and cherry, lowering water permeability (Benckiser 2019). Using zeolites or nanoclays in the soil improves water absorption in plants (Duhan et al. 2017). When nanoceria, highly charged NPs which are reactive oxygen species (ROS) scavengers and SWNTs are introduced to the mesophyll tissue, they localize inside the chloroplasts, increasing photosynthesis and the lifespan of extracted chloroplasts (Giraldo et al. 2014). For the treatment of plant illnesses as well as the discovery of chitosan's cellular localization and immunomodulatory role in plants, chitosan nanoparticles (CNP) are a more effective therapeutic tool. The exact imaging-based visual portrayal of the relative bio-accessibility and bio-accumulation of chitosan versus its NPs (CNP) in plant cells reveals that CNP interacts with plant cells at a higher level than was noticed for natural chitosan (Chandra et al. 2015).

In general, NP-mediated biomolecule distribution is less effective than traditional biotic delivery methods like agro-infiltration, though it may be necessary in specific cases (due to species). Commercial transfection agents are costly, thus they can't be applied widely (Sarker et al. 2020). Lower efficiency cannot be overcome by simply increasing the amount of delivered NPs due to toxicity. Thus the interactions between macromolecules must be studied to understand which NPs and with what concentration must be applied (González-Grandío et al. 2021). The importance of size was demonstrated when Zhang and coworkers non-biolistically delivered DNA coated onto gold NPs of spherical and rod-like shapes, between sizes 5–20 nm into

Nicotiana benthamiana L. leaves. The smaller NPs showed interaction with plant cell walls occurs at higher, more frequent, and more persistent levels. However, only the rod shaped ones were internalized by the cells. Yet, it was also observed that small-interfering RNA (siRNA)-which were functionalized on the 10 nm spherical AuNPs were the most effective for delivery and they induced gene silencing in the leaves. The work also showed that internalization is not required for the RNA-NP interaction to be efficient for nucleic acid delivery (Zhang et al. 2021).

GO shows high ecotoxicity. Low levels of graphene promote the growth of seedlings, but large levels of the material lead to oxidative stress, cell damage, decreased protein synthesis, and genetic alterations. By obstructing seed epithelial pores, graphene in rice limits the amount of water in seeds (Zhang et al. 2015). Rice seedling stem development is severely impeded due to the blockage of plant growth factor synthesis or excessive enzymatic activity. As carbon nanotubes puncture seed epithelia, forming new nanoscale channels, or as a result of their capillary action being similar to water channel proteins, other seedlings displayed increased water content and germination rates. Metabolic by-product levels rise later and prevent graphene invasion to safeguard seedlings. Static electricity allows low concentration GO to stick to the surfaces of roots. An increase in membrane oxidation caused by a moderate dose can lead to aberrant biological markers and uneven gene levels. Plants may be impacted by physical obstruction or mechanical harm by changes in reactive oxygen species (ROS) (Yang et al. 2020).

The following table shows how different concentrations of the same NP can cause different morphological and physiological changes in various plant species (Table 9.2).

9.6 Interaction of NPs with Nucleic Acids

9.6.1 Interaction of NPs with RNA

9.6.1.1 Interaction with Carbon Nanotubes

siRNA can be loaded onto SWNTs by probe-tip sonication. siRNA desorbs from SWNT carriers only in the cytosol, as proven by thermodynamic analysis of hydrogen bonding and π-π stacking interactions energetics. 98% of free-siRNA is degraded by RNases inside the cell, and only 16% of SWNT-bound RNA is, showing that it can give stability within the cell (Demirer et al. 2019).

Table 9.2 Effect of diverse nanomaterials on plant physiology

Nanomaterial	Size/Concentration	Plant material	Effect	Reference
Carboxylated COOH-SWNTs	50 mg/L	*Solanum lycopersicum* L. (tomato)	Induced growth	Khodakovskaya et al. (2010)
CdS quantum dots	200 mg/L	*Tohya* variety soybean	Oxidative stress and root lignification	Majumdar et al. (2019a, b)
CeO_2 NPs + Sb (III)	0.075 wt%	*Oryza sativa* L. (rice) Roots and shoot	Synergistic stimulation by 68.30% and 73.48% respectively	Cao et al. (2022)
Fe_3O_4	30 nm, 100 mg/kg	*Zea mays* L. (maize)	Increased leaf biomass by 15%	Zhao et al. (2019)
	1000 mg/kg	*Arachis hypogaea* L. (peanut)	Increased biomass and chlorophyll	Rui et al. (2016)
Graphene	2000 mg/L	Cabbage, tomato, spinach and lettuce seeds	Inhibited growth and reduced biomass	Begum et al. (2011)
Graphene oxide	25- to 100-mg/L	*Brassica napus* L. (rapeseed)	Inhibits root growth	Cheng et al. (2016)
Multi walled nano tubes	1000 mg/L	*Triticum aestivum* L. (wheat)	Enhanced the germination and seedling root elongation	Miralles et al. (2012)
	1000 mg/L	*Onobrychis arenaria* (Kit.) (legumes)	Stimulated the growth of roots and stems	Smirnova et al. (2012)
Multi walled carbon nanotubess	1000 ppm	*Cucurbita pepo* L. (zucchini)	60% decrease in dry weight	Stampoulis et al. (2009)
Nano-TiO_2	5 nm	*Spinacia oleracea* L. (spinach) seeds	Accelerates germination	Zheng et al. (2005)
	50 mg/kg	*Lactuca sativa* L.	Increases shoot dry weight and length	Zahra et al. (2015)

(continued)

Table 9.2 (continued)

Nanomaterial	Size/Concentration	Plant material	Effect	Reference
	20 nm	*Triticum aestivum* L. *var.* Pishtaz (wheat) grains	No effect	Feizi et al. (2011)
	100 mg/kg	*T. aestivum* L. (wheat)	Root length and biomass	Rafique et al. (2014)
Nano-ZnO	20 nm, 2000 mg/L	Rapeseed, radish, ryegrass, lettuce, corn and cucumber	Inhibits root growth	Lin and Xing (2007)
Non-zero valent iron	Till 4.18 ppm	*Arachis hypogaea* L. (peanut)	Increases root growth	Tolaymat et al. (2017)
Poly-3-aminobenzenesulfonicacid SWNTs	1750 mg/L	*Solanum lycopersicum* L. (tomato)	Inhibitory to roots	Cañas et al. (2008)
Single-layer graphene	50 mg/L	*Oryza sativa* L. (rice) seeds	Represses germination rate	Zhang et al. (2015)
	100 mg/L	*Oryza sativa* L. (rice)	Restrict root and stem length, fresh weight, root-shoot ratio	Zhang et al. (2015)

9.6.1.2 Interaction with BioClay

Layered double hydroxide (LDH) nanosheets are synthesized as stacks of positively charged sheets of size 15–120 nm. LDH nanosheets are inorganic layered materials, given by the formula:

$[(M^{2+}{}_{(1-x)}M^{3+}{}_{x}\ (OH)_2)^{x+} \cdot (A^{m-}{}_{x/m} \cdot nH2O)^{x-}]$, M indicating metal ions and A for anions (Xu et al. 2005).

By loading dsRNA into LDH in a mass ratio of 1:4, dsRNA-LDH complexes (BioClay) are created, which no longer move during electrophoresis, demonstrating neutrality owing to binding. The dsRNA chain may be seen in part by TEM imaging between LDH nanosheets, indicating that it is either adsorbed on the surface or wrapped around several LDH particles (Mitter et al. 2017). Atmospheric CO_2 and moisture break down LDH slowly into a biocompatible residue, releasing the loaded biomolecules (Xu et al. 2005). When droplets of LDH suspension were placed on detached tobacco leaves at 27 °C in 95% relative humidity and 5% CO_2 for 7 days, the LDH residue had 28% decrease in aluminum and 22% decrease in magnesium respectively. In solution, when dsRNA–LDH is suspended for a week in 5% CO_2, northern blot reveals a reduction in residual LDH-bound dsRNA compared to normal atmospheric CO_2 conditions. Spraying the loaded NPs on leaves still permits detection of abundant levels of the conjugates even after 30 days, indicating protection of nucleic material from RNase. The complexes also dissociate at pH 3.0. Carbonic acid formation on the leaf surface causes a slow and sustained release of dsRNA. RNase treatment degrades naked dsRNA more than the conjugate. Its application is in protection of plants from RNA viruses (Mitter et al. 2017) (Fig. 9.4).

9.6.2 Interaction of NPs with DNA

9.6.2.1 Interaction with SiNPs

Functionalized-SiNPs (f-SiNPs) interact with DNA at $H_2PO_4^-$ through ionic bonds and have a stronger affinity for the cells. To test for NP interaction, DNA from leaves of the *Bixa orellana* L. plant was employed. A260:A280 ratios greater than 1.8 are considered to be indicative of protein and RNA contamination, according to spectrophotometry. SiNPs interaction resulted in the reading of a value of 1.802. SiNPs and f-SiNPs denature into single-stranded DNA or intercalate into DNA double helices by hydrophobic contact, which may then contribute to further DNA replication. Due to its hydrophobicity, which protects the DNA, the alanine functional group exhibits greater intercalation. DNA is denaturalized or replicated by nanoparticles with lysine functionalization (Kumar et al. 2021).

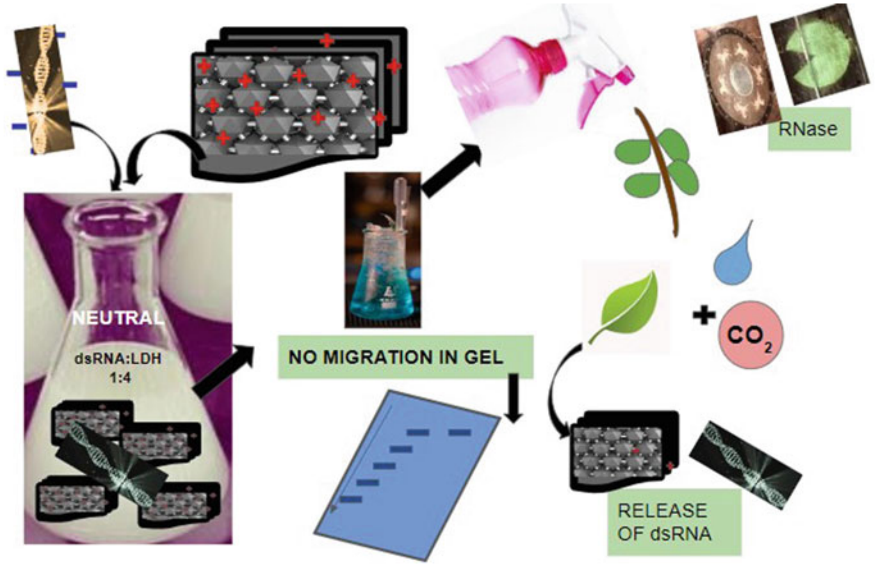

Fig. 9.4 BioClay preparation and application (Elements in this image have been created with the help of Pixabay. https://pixabay.com/)

9.6.2.2 Interaction with Arabinoxylan

Polymer complexation and nanoprecipitation by solvent shifting create the self-assembled arabinoxylan nanostructures. They were dissolved in distilled water with CRISPR-Cas9 vector DNA for 30 min at room temperature for binding. Characterization by Dynamic light scattering (DLS) at 90° angle showed that the free particles form highly monodisperse NPs, the average hydrodynamic diameter of anionic NP being 93.25 ± 19.24 nm and of the cationic NP being 125.08 ± 25.83 nm. Monodispersed means that the colloidal stability of NP aggregation is not influenced by water, but rather by surface charges and steric effects. Large, unstable colloids formed when the cationic and anionic AX electrostatically complexed. The cationic AX also forms stable complexes with the CRISPR-Cas9 vector DNA, at a ratio of 15:1. When analyzed by TEM, the particle size is smaller than in DLS due to the hydrophilic corona shrinking from drying (Sarker et al. 2020).

9.6.2.3 Interaction with SWNTs

Carbon nanotube scaffolds are coated with sodium dodecyl sulphate (SDS) surfactant and then subjected to dialysis in order to transplant DNA. SDS will desorb, and by dynamic ligand exchange process and π-π stacking interactions, DNA adsorbs onto the surface of carbon nanotubes. A distinctive solvatochromic change in the SWCNT nIR fluorescence emission spectra in the CNT dielectric environment can

Table 9.3 Variation of physical properties of nanomaterials due to functionalization

Verification	Atomic force microscopy: (Height)	Zeta potential
COOH-Single walled nano tubes	1.3 nm	−51.9 mV
PEI-Single walled nano tubes	8.1 nm	+40.2 mV
DNA-PEI-Single walled nano tubes	16.3 nm	+31.7 mV

serve as evidence of adsorption. Carboxylated CNTs (COOH-CNT) are covalently modified with a cationic polymer (polyethylenimine, PEI) for a net positive charge in the electrostatic grafting process. After that, they are incubated for 30 min with negatively charged DNA vectors in a ratio of 1:1 by mass (Demirer et al. 2019). The following table shows the change in the physical parameters with each step in grafting of DNA to the NP (Table 9.3).

When it comes to loading DNA, electrostatic grafting is 100% more effective than the dialysis technique (50–70%). The electrostatic attraction has smaller equilibrium dissociation constant and a greater binding energy value than π-π—stacking interactions. By incubating the conjugates with proteins that mimicked plant intracellular conditions, the stability of the conjugates was demonstrated. Half of the DNA remained adhered to the carbon nanotubes after 3 days. It is also possible to store PEI-CNTs for a month at 4 °C (Demirer et al. 2019).

9.6.2.4 Interaction with DNA Material

There are some negative interactions of NPs with DNA. The interaction between the surface DNA-wrapped carbon nanotube and lipids decreases water density around the nanotubes (Jena et al. 2017). Tan et al. (2009) showed that 20, 40 and 80 ppm of 10–30 nm MWCNT causes chromatin condensation and shrinkage of cells in *O. sativa* L. (Tan et al. 2009). DNA was damaged when *R. sativus* L. and *L. perenne* L. were exposed to 10, 100, 500, and 1000 ppm of 100 nm size copper oxide NPs (Atha et al. 2012). Fullerene disturbs the routes of electron transport and energy by suppressing transcriptional genes (Hossain et al. 2016).

When carbon nanotubes pass through water channels of *Solanum lycopersicum* L., they activate the aquaporin gene (LeAqp2) and genes related to stress (Khodakovskaya et al. 2010). Damage to structural DNA is seen in *G. max* L. upon 2000 and 4000 ppm exposure of engineered CeO_2 NPs (López-Moreno et al. 2010). 4 mM of TiO_2 NPs caused bridge formation in telophase and anaphase of *A. cepa* L. (Ghosh et al. 2010). TiO_2 NPs at 2 and 10 mM concentration damaged DNA in *N. tabacum* L. and *Z. mays* L. respectively. They also cause fragmentation of chromosome arms in narbon bean (*Vicia narbonensis* L.) (Ruffini Castiglione et al. 2010). 100 mg/kg of soil of Fe_3O_4 also particularly inhibited pyrimidine metabolism in maize, which could be a strategy to conserve resources for stress-related processes as pyrimidines are mainly used in reproduction (Kafer et al. 2004).

Aside from these, interactions of DNA and NPs can have important applications. Gold NPs were functionalized with a specific single-stranded DNA and could detect just 15 ng of *R. solanacearum* Smith genomic DNA, to assay a soil bacteria that causes potato wilt (Khaledian et al. 2017). With a synergistic interaction between Tb(lll) ions and CDs, a paper-based fluorescent Tb(lll)-CD probe was created to detect as little as 50 nM of 3′-diphosphate-5′-diphosphate, the reaction of plants to harsh environmental circumstances (Chen et al. 2018). Different genes and pathways can be up or down regulated upon interaction with nanomaterials. A recent report shows that under Co-induced stress conditions, nanomaterial fullerenes control the gene expression of the RuBisCo large subunit (rbcL), tonoplast intrinsic protein2-1 (TIP2-1) and nodulin 26-like intrinsic protein1-1 (NIP1-1) (Ozfidan-Konakci et al. 2022). A study by Pagano et al. (2022) showed how treatment of *Arabidopsis thaliana* L. with nanoscale FeO_x and ZnS QDs increase in plastid (pt) and mitochondrial (mt) DNA copy numbers by 1 to 3 times. The following table describes how diverse NPs interacting with nucleic material affect the metabolism of the plant (Table 9.4).

Metal oxide NPs are thought to release ions on entering the cell, which may have their own impact depending on the metal's chemical properties and the overall crystal structure of the particle. Genes can be affected in different ways depending on the NP. The following table describes when a gene gets up or down regulated in response to which NP (Table 9.5).

SRO5 is upregulated for a variety of NPs but not CeO_2 (Khandelwal et al. 2008). *A. thaliana* L. from Ac/Ds mutagenized lines are resistant to normally lethal concentration of CdS quantum dots (Marmiroli et al. 2014). The genes for leucine biosynthesis: IMD3, IPMI2 and IPMI1, and for fatty acid metabolism (FAD3), are strongly down-regulated.

9.6.2.5 Interaction with Carbon Nanotubes

Biomolecules bound to carbon nanotubes are protected from cellular metabolism and degradation (Wu et al. 2008). Oligonucleotide adsorbed-pristine SWNTs and polyethyleneimine-SWNTs loaded with plasmid DNA (PEI-SWNTs) are used for plant delivery and sensing applications. This gives transient expression, regardless of plant species, which can be confirmed by ddPCR experiments. Plasmid DNA delivered by PEI-SWNT is 700 times more efficient than the pristine nanomaterial and linear DNA. This is probably due to plasmid DNA being unaffected by exonucleases, while linear is affected by both exo- and endonucleases present in the plant cell. Protoplast transformation was 76% efficient when the nuclear localization signal UBQ10 was used and 86% when 35S was used. When tobacco, arugula, wheat, and cotton leaves were treated with plasmid DNA-PEI-SWNT conjugates, there was around 7500-fold expression 3 days after infiltration, and just two-fold on the tenth day, indicating transient expression. There is no decrease in quantum yield measurements of photosystem II in tobacco leaves, showing non-toxicity (Demirer

Table 9.4 Interaction of plants and nanomaterials leading to change in gene transcription

Nanomaterial	Size/Concentration	Plant part	Gene types	Effect on gene	Reference
Ag NPs	0.5 and 1 mg/ L	*O. sativa* L. seedling	MSD1, CSD1, FSD	Tolerance of oxidative stress	Nair and Chung (2014)
Multi walled nano tubes	50 mg/L	*Solanum lycopersicum* L. (tomato) roots	Water-channel proteins and hormone pathways	Upregulation	Khodakovskaya et al. (2010)
Multi walled carbon nano tubes	100 µg/mL	*Nicotiana tabacum* L. (tobacco)	NNtPIP1-induces aquaporins NtLRX1-cell wall formation CycB- division	Upregulation	Khodakovskaya et al. (2012)
Nano-TiO_2	20 mg/L	*Arabidopsis thaliana* L	Phosphate-starvation, root-development	Repression	García-Sánchez et al. (2015)
Nano-ZnO	100 mg/L	*Arabidopsis* roots	Biotic and abiotic stress responses	Up-regulation	
			Cell biosynthesis, electron transport, and energy pathways	Down-regulation	Landa et al. (2012)
Single walled nano tubes	8.1 nm length	*Nicotiana benthamiana* Domin (benthi) leaves	NbrbohB	No upregulation, nontoxic	Demirer et al. (2019)

Table 9.5 Effect of diverse NPs on different genes

Gene	Description	CdS QD	Ag NPs	ZnO	CuO	TiO_2	Reference
CHL	Chloroplastic lipocalin, oxidative stress response	Up regulation	–	–	Down regulation	Up regulation	Malnoë et al. (2017)
FSD1	Fe-Superoxide dismutase, oxidative stress and to bivalent ions response	–	–	Down regulation	Down regulation	–	Perea-García et al. (2015)
NIR	Reduction of nitrite, salt stress	Down regulation	–	Down regulation	Down regulation		Garai and Tripathy (2018)
SRO5	ROS scavenging and salt stress	Up regulation	Up regulation	Up regulation	Up regulation	Up regulation	Khandelwal et al. (2008)

et al. 2019). However, contradicting results were seen in *Arabidopsis* by RNA-seq analysis of leaf infiltrated with 50 mg/L SWNTs and PEI-SWNTs, 48 h after exposure. The conjugates are transported across the plant nuclear membrane and they dissociate in the nucleoplasm for gene expression. Pristine SWNTs are well tolerated by the plant since they trigger a modest stress response with no phenotypic change, comparable to the water infiltration process. However, PEI-SWNTs led to transcriptional reprogramming, which was akin to senescence's constitutive immune response or what happened when plants were exposed to stress-priming agrochemicals. This indicates that PEI causes biotoxicity (González-Grandío et al. 2021). Table 9.6 shows the number of genes whose transcription is affected when infiltrated only with water and when exposed to the NP. It is possible to obtain the gene panel of those genes that are affected only by SWNT infiltration by removing the ones common to it and water. The same can be done to find the gene panel for PEI effect (Table 9.6).

Table 9.6 Magnitude of transcriptional change due to infiltration and nanomaterials

Material infiltrated	No. of upregulated genes	No. of down-regulated genes
Water	452	321
Single walled nano tubes	797	347
Polyethylenimine single walled nano tubes	1364	997

Hypoxia, which can be brought on by injecting a liquid into leaf tissue with lots of air gaps, was linked to genes that were common to all pathways. Other prevalent genes were involved in the construction of cell walls and the manufacture of glycosinolates (defense metabolites). Therefore, hypoxia activates additional stress-related genes. Between the SWNTs and PEI-SWNTs treatments, there are 76% of upregulated and 62% of downregulated genes in common. Therefore, independent of the surface functionalization, these genes are responsive to carbon nanotubes. The immune system, cell death mechanisms, and the manufacture of the principal aromatic amino acid (tryptophan) and secondary metabolite (salicylic acid) are all overrepresented in the genes of PEI-SWNTs treated leaves. This is comparable to plants that are dehydrated (Tran et al. 2007) and opposite to those of plants treated with glucose (Li et al. 2006). Due to PEI-SWNT treatment, the genes PCR2 for Zinc detoxification, FRK1 for senescence, and PR1, CHX17 and PAD3 for leaf chlorosis and cell death are upregulated. The genes AGP41, At3g54830 and NAI2 which are also related to leaf chlorosis and cell death are down regulated.

In plants, a prolonged immune response often leads to programmed cell death (Pitsili et al. 2019). Because of the polymer's tiny size and reduced amine density, hydrophobically modified low molecular weight linear L-PEI (800 Da) is tolerated by *Nicotiana*. PEI-750 k's low toxicity to plants may be a result of its size, which hinders cellular internalization. This temporal response could prepare plants to more effectively withstand upcoming abiotic challenges because low levels of PEI-SWNTs could be sustained for several days.

9.7 Interaction of NPs with Proteins

The study of proteomics is done by separating proteins based on their isoelectric point, mass and hydrophobicity, after which mass spectrometry for fingerprinting is conducted (Pagano et al. 2018). NPs have weak molecular reactions with proteins through Van der Waals' forces, static electricity, hydrogen bonding and hydrophobic interactions, which make alterations in protein structure and function (Lynch and Dawson 2008).Upon entrance of metal NPs into the root cells, they get dissolved into redox-active ions which interact with protein functional groups like carboxyl and sulfhydryl, altering their activity (Hossain et al. 2020). The ions generate ROS by Fenton and Haber–Weiss reactions (Halliwell and Gutteridge 2015). Metal ions react with hydrogen peroxide, forming hydroxyl radicals and anions which are even more toxic ROS. Quinone reductases (QRs) of the plants detoxify the free radicals. When zinc oxide and silver NPs are applied to soybean seedlings, a severe oxidative burst causes their levels to decrease along with thioredoxins (Hossain et al. 2016).

A bio-corona can arise when proteins and other macromolecules adsorb on nanomaterials (González-Grandío et al. 2021). When under Co stress, nanomaterial fullerene causes ascorbate regeneration in *Zea mays* L., through increasing monodehydroascorbate reductase and dehydroascorbate reductase levels (Ozfidan-Konakci et al. 2022). When *A. thaliana* L. is treated with 5.8 μM quantum dots, there is a drop in glutathione (Navarro et al. 2012). Maize grown in 100 mg/kg soil treated with Fe_3O_4 had increased concentrations of the amino acids aspartic acid, lysine, serine, valine as well as phenylalanine, a precursor of antioxidant phenolic acids in the leaves, and tyrosine in the roots (Zhao et al. 2019). Concentrations of polyamines, like spermine and its citrulline precursor, implicated in the reaction to stress and the development of diverse plant tissues were increased (Alcázar et al. 2010). The polyamine putrescine was produced in response to both TiO_2 and Fe_3O_4.

Soybean plants were treated with 200 μg/ml cadmium sulfide-quantum dot (CdS-QD) treated vermiculite. Transport across the roots is aided by a phosphate transporter and a copper-binding transmembrane metal transporter. HIPP22 genes that regulate Cd binding protein themselves show reduced expression in a negative feedback loop. ATPase-coupled Ca^{2+}-transmembrane transporter protein (K7LC34) is also expressed less, preventing transport of copper to the leaves. These were coated with thiol mercaptoacetic acid (MAA) and glycine (GLY) which accumulated in root cell walls. Coating of hydrophobic trioctylphosphine oxide (TOPO) caused instability and released Cd^{2+} into the cell membranes. This down regulated protein transport channels so less influx of Cd would take place to the cytosol and chloroplast. Water-soluble polyvinylpyrrolidone coated QD was transported to the leaves where it reduced biomass. These last 3 had the largest hydrodynamic diameters. Tandem mass spectrometry was used to analyse trypsin-digested protein. Zeta ζ-potential values in root exudate medium ranged from -24 to -28 mV for all the coatings except for the TOPO coat which had -17.5 mV. These values were all less than that for Milli-Q-water, showing the increased stability due to root metabolites forming a biocorona around them. Several proteins were found to be expressed in roots only due to the treatment with QDs. These affect metabolic pathways of glutathione, carbon, amino acids and secondary metabolites like isoflavonoids and monoterpenoids. The defensive response, ion complexing, membrane organization and channel dynamics are additional functions they play. Tryptophan is accumulated to over thrice the normal concentration. Calcium-transporting ATPase activity however was downregulated. There was also less of defense enzymes like peroxidases, cytochrome P-450, phenylpropanoid pathway intermediates like coumarinate and caffeoyl CoA. Proteins that accumulate in the roots due to Cd^{2+} stress include those for Cys biosynthesis, TCA cycle, carbon fixation and glyoxylate metabolism (Majumdar et al. 2019a, b). Proteins for β-oxidation and phenylpropanoid pathways, lignin, sphingosine and jasmonic acid biosynthesis are over abundant in the CdS-QD-treated roots as a result of stress signaling pathway overexpression (Sharma et al. 2019).

9.7.1 Interaction with Specific Proteins Dealing with Stress

Excessive NaCl levels were used to culture the “Valencia” (*Citrus x sinensis* L. Osbeck) sweet orange plants, which led to salt stress, foliar drop, and reduced growth. When 400 mM of nanosized, monodisperse silicon particles were sprayed directly onto the leaves, the water status, ion content regulation, root growth, photosynthesis, chlorophyll content, and osmotic effect were all improved. Additionally, it increased the amount of aquaporin (CsPIP1;1, CsPIP2;3, and CsTIP4;1) and Na^+ cotransporter (CsSOS1, CsSOS2, CsSOS3, and CsNHX1) in the root tissues (Mahmoud et al. 2022). Crops commonly suffer from boron deficiency, and boron has limited mobility inside plant tissues. This micronutrient was encapsulated in nano-proteoliposomes made from natural membranes as a delivery mechanism. *Ipomoea batata* L. plants grown in vitro were used to evaluate this method, and they demonstrated a ten-fold rise in boron in the leaves as well as increases in Fe and Mn. Additionally, noticeable increase in formic acid, asparagine, and valine was observed. Boron transporters (BOR2, BOR4, and BOR7;1) and NIP aquaporins (NIP1;2, NIP1;3, NIP4;1, NIP4;2, NIP5;1, NIP6;1, and NIP7) are also expressed more (Nicolas-Espinosa et al. 2022). In the mitochondria, ammonium and fatty acid transporters play major roles in detoxification of NPs and are also the target of nanotoxicity. TiO_2, ZnO and CuO cause the modulation of AlaAT1, an alanine aminotransferase, which usually responds to conditions of hypoxia. Cold temperatures sensitive MDAR6, a monodehydroascorbate reductase, mitochaondrial encoded mtLPD1, mtLPD2 lipoamide dehydrogenases and mtHsc70-1 heat shock protein, are also affected. All of the mentioned genes respond to cadmium exposure. HSP60 is a mitochondrial protein with functions of organization, heavy metal and heat shock response. SAMC1 is an S-adenosylmethionine transmembrane transporter for organelles. In *A. thaliana* L., HSP60 and SAMC1 are up-regulated upon exposure to TiO_2NPs. HSP60 is down-regulated with ZnO (Pagano et al. 2018). Cerium oxide NPs caused increase in HSP70 in maize, as well as increased ascorbate peroxidase and catalase (Zhao et al. 2012). In the chloroplast, treatment of 10 mg/L silver NPs on *Spirodela polyrhiza* L. reduced RuBisCo activity by 37% (Jiang et al. 2017), and inhibited Photosystem II. In soybean roots treated with zinc oxide NPs, gel-free proteome analysis revealed a reduction in redox cascade proteins such as galactose oxidase, GDSL motif lipase 5, quinone reductase and SKU5 similar 4 (Hossain et al. 2016). The interaction of soybean plants with CdS NPs with different functional groups caused the differential increase or decrease of the proteins peroxisome-localized uricase-2 isozyme-1, Pectin esterase, γ-GST, glutamate dehydrogenase, amine oxidase, γ-glutamyl hydrolase, 3Fe-4S cluster binding proteins, phosphoric diester hydrolase, GST, carboxypeptidase, GSH synthetase, S-(hydroxymethyl) glutathione dehydrogenase, SOD, Pathogenesis related gene (PR1), MT type-2B, SULTR4;2 and TIP2;1 (Majumdar et al. 2019a, b).

9.7.2 Interaction with Specific Proteins Dealing with Effect on Growth

In general, changes in the activity of particular enzymes directly correspond to the level of stress experienced by plant cells after being externally stimulated through stress, which leads to the breakdown of the equilibrium system. SOD and POD are antioxidant protection enzymes that aid in the body's ability to deal with oxidative malfunction and maintain the ever changing equilibrium of ROS, according to several studies. While SOD can capture superoxide ion free radicals throughout the growth of the organism, regulate the membrane structure, and limit ROS generation, POD primarily catalyses the breakdown of hydrogen peroxide into oxygen and water. The H_2O_2 produced by SOD, catalase, and POD is quenched by nanomaterial fullerenes in *Zea mays* L. (Ozfidan-Konakci et al. 2022). Treating *Ruta graveolens* L. with 0.1 μM and 2.5 μM of CuO NPs increased the total protein levels, but decreased SOD. 0.1 μM concentrations could increase CAT activity, while 2.5 μM decreased it in the shoot. Both concentrations increased shoot IRT1 expression (iron-regulated transport 1), which is important for Mn, Zn, Cd, and Fe transport and essential for iron homeostasis (Mazaheri-Tirani et al. 2021). Additionally, MgONP treatment led to significant POD activities in tobacco plants, which were able to remove too much H_2O_2 from cells, maintain membrane integrity and reduce the peroxidizing effects of H_2O_2 on its lipids. These outcomes might affect how POD activity is stimulated and how oxidative stress is reduced. Raised POD and SOD activity, unaltered levels of MDA, and protein levels, and rather constant water levels may show that the NPs heightened the plants' oxidative stress while sparing the tobacco plants' membranes any harm (Cai et al. 2018). CNP and chitosan bind to cells extracellularly. Chitosan NPs (CNP) can be used at a dose almost ten times lower than that needed for chitosan. CNP may similarly function as a powerful plant defence inducer in a cellular setting and achieve significantly higher immunomodulatory efficiency. CAT and SOD, two essential antioxidant enzymes involved in the scavenging of ROS, were shown to have considerably greater enzyme activity and mRNA expression levels in the chitosan and CNP treated *Camellia sinensis* L. Kuntze leaves compared to the untreated control counterparts. This outcome is in line with that of Ortega-Ortez et al. who demonstrated that tomato fruits can exhibit CAT activity when chitosan and salicylic acid are applied (Ortega-Ortiz et al. 2007). The CNP-treated leaves had larger accumulations of peroxidase (PO), polyphenol oxidase (PPO), beta-1, 3-glucanase, and phenylalanine ammonia lyase (PAL) than their chitosan-treated counterparts. It is known that PO and PPO participate in the lignin production pathway. By increasing the plant's cell wall barrier, this pathway improves defense against several diseases and pathogens. Induced production of beta-1, 3-glucanase would also improve cell division, flower development, and seed maturation. Therefore, because chitosan or CNP treatment can directly affect the hydrolysis of glucans in fungal cell walls, it may be useful to induce the expression of beta-1, 3-glucanase at the moment of pathogen invasion. Enhanced thaumatin like protein (TLP) activity could improve immunity to diseases, such as those vectored by insects. The phenylpropanoid biosynthesis pathway's initial enzyme, PAL, is also involved in the creation of phenolic compounds and phytoalexins. It has already been established that phenolic compounds play a role in disease resistance. Tea leaves treated with CNP accumulated more phenolic compounds than tea leaves treated with chitosan, which may have been caused directly by the PAL activity that was increased in the CNP-treated portions. The analysis of the induced expression of the

C4H, ANR and F3H genes in the chitosan- and CNP-treated leaves confirmed this up-regulation. The flavonoid biosynthesis pathway, which involves F3H, produces a broad family of flavonoid molecules with a variety of biological properties, including disease resistance. In addition, F3H catalyzes the biosynthesis of other flavonoids. These act as feeding inhibitors and as a potent antioxidant system. Plants treated with chitosan and CNP produced more NO than the untreated (Chandra et al. 2015). The concentration of GO determines the effect on plant growth. Table 9.7 gives the effect of GO on rapeseed. For this cash crop, the critical stimulating effects of GO sheets may take place between concentrations of 10–25 mg/L (Yang et al. 2020).

NPs can also behave like antioxidants: nCo_3O_4, nFe_3O_4, and $nCeO_2$ imitate CAT; nCo_3O_4, $nCeO_2$, nFe_3O_4, nAu, $nMnO_2$ and nCuO and imitate peroxidase; $nCeO_2$, fullerene and nPt imitate SOD (Wei and Wang 2013).

9.7.3 Applications of NP Interaction with Proteins

CdSe/ZnS core–shell QDs tagged with anti-gliadin antibodies were used to analyze the distribution of gliadin during baking (Ansari et al. 2015). These conventional QDs can have autofluorescence and photobleaching, so they are doped with lanthanides to create fluorophores. Ricin can be detected by amine-functional europium-doped KGdF4 NPs.

The absorption property of MWCNTs is high owing to the more expanded surface area, chemical and thermal stability. GO planar sheets have better adsorption than MWCNTs due to ease of synthesis from graphite, and the latter's steric hindrance for compounds, and metal contamination (Liu et al. 2011). They are effective energy acceptors with delocalized electronic excitations for long-range resonance energy transfer (LrRET) which quenches fluorophore emission. GO quenches fluorescence and amplifies fluorescence anisotropy (FA) due to its larger mass and slower rotation rate (Xiao et al. 2015).

During protein detection, antibody probes interact better with the target in the presence of nanomaterial coated electrodes. Aptamers are nano-sized receptors for proteins, which have been used in apta assays to detect gliadin in food by binding to its immunodominant apolar peptide. Stem-loop DNA aptamer probes can capture toxin genes and undergo a conformational change which changes the effectiveness of electron transfer, creating an electrochemical signal (White et al. 2016). QD-aptamer-GO NPs change shape when interacting with food allergens, causing difference in fluorescence caused by quenching and signal recovery by GO (Weng and Neethirajan 2016). To prevent this quenching, space should be there between the fluorophore and GO.

The lanthanide doped NPs used GO as a ricin competitor to adsorb the aptamer and quench the excited donor fluorophore (Huang et al. 2014). On recognizing ricin, the aptamer becomes a rigid tertiary structure which gives only partial fluorescence as it cannot be bound and quenched, so it is used for detection. A biosensor detects Ara h 1 peanut allergen using DNA probes immobilized on a glassy carbon electrode

Table 9.7 Regulation of plant protein expression based on nanomaterial concentration

Nanomaterial concentration	Plant	Plant proteins	Regulation	Observation	Reference
25- to 100-mg/L of GO	*Brassica napus* L. (rapeseed)	SOD	Up regulated	Inhibits root growth, Lipid peroxidation increases slightly	Cheng et al. (2016)
		Catalase	Up regulated		
		POD	Down regulated		
5–10 mg/L of GO	*Brassica napus* L. (rapeseed)	SOD	Down regulated	Stimulates root growth	Cheng et al. (2016)
		Catalase	Down regulated		
		POD	Up regulated		
20 mg/L GO	*Solanum lycopersicum* L. (Wild tomato)	SOD	Down regulated	Root length increases	Jiao et al. (2016)
		POD	Down regulated		
		CAT	Down regulated		
100, 200, 1600 mg/L	*Vicia faba* L. (broad beans)	APX	Down regulated	Germination, root extension inhibited, increased ROS	Anjum et al. (2014)
		CAT	Down regulated		
400, 800 mg/L	*Vicia faba* L. (broad beans)	APX	Up regulated	Enhanced growth, more water content, less ROS stress, less electrolyte leakage	Anjum et al. (2014)
		CAT	Up regulated		
Aluminum oxide NPs (8 nm)	*Nicotiana tabacum* L.	Dehydrogenase,	Down regulated		Poborilova et al. (2013)
		Oxido-reductase	Down regulated		
NiO NPs	*Solanum lycopersicum* L. (Tomato)	SOD	Up regulated	Lipid peroxidation increases, more glutathione content	Faisal et al. (2013)
		CAT	Up regulated		

(continued)

Table 9.7 (continued)

Nanomaterial concentration	Plant	Plant proteins	Regulation	Observation	Reference
nTiO$_2$-A	*S. oleracea* L.	SOD	Up regulated		Lei et al. (2007)
		CAT	Up regulated		
		APX	Up regulated		
		GPX	Up regulated		

APX = Ascorbate Peroxidase
SOD = Superoxide dismutase
POD = Peroxidase (Horseradish roots) enzyme

(GCE) with chitosan MWCNTs which promotes kinetics of electron transfer (Sun et al. 2015). Au NP-coated screen-printed carbon electrode conjugated with monoclonal antibodies was used as a voltammetric biosensor for the detection of peanut allergens Ara h 1 and Ara h 6 in food (Alves et al. 2015a, b). A detector complex of Surface-enhanced Raman scattering (SERS) platform is created by attaching optimized single-stranded oligodeoxynucleotides (ssODNs) to Au NPs (Tang et al. 2016). It is used for the detection of ricin which depurinates the ssDNA releasing adenine, which causes signal attenuation. Silica nanospheres and gold film-over-nanospheres create a SERs platform where a glycopolymer NAGEMA is attached to detect ricin B-chain in spiked fruit juice (Szlag et al. 2016).

Nanomaterials of gold, copper and cobalt have high conductivity, adsorption due to extensive surface area, and biocompatibility, so they are used in electrochemical sensors. Silver NPs have high fluorescence enhancement due to radiative decay engineering and local field enhancement. Silver NPs of diameter 50 nm created dendrites for detecting ricin in liquid food samples using SERS spectrum (He et al. 2011). Carbon, silica and metals give structural support to sensors by linking antibodies with probes or modifying the electrode surface.

Ammonium persulphate is used to modify canola protein which are attached to graphite oxide (GO) and nanocrystalline cellulose (NCC) to create a naturally sourced hybrid adhesive. The former is in the form of single or stacked sheets that range in width from 600 to 800 nm. The latter has a fibrous rod structure of diameter 60–90 nm. Ammonium persulphate crosslinks the protein by inter tyrosine and tyrosine-histidine free radical interactions creating a protein network that is covalently stabilized. The hydrophobic functional groups, nanomaterial exfoliation, cohesive interactions and thermal stability cause the adhesive to have improved properties like water resistance (Bandara and Wu 2017).

NPs are used for the treatment of plant viruses. Positively charged LDH sheet-like clay NPs are used as dsRNA carriers to protect against plant viruses (Mitter et al. 2017). *N. benthamiana* Domin and cowpea (*Vigna unguiculata* L.) could resist aphid vectored potyvirus, bean common mosaic virus (BCMV), 5 days after topical application of dsRNA using BioClay. Silver NPs (AgNPs) bind the coat proteins of tomato mosaic virus (ToMV) and potato virus Y (PVY) in plant (El-Dougdoug et al. 2018). 50 mg/L spray reduced local lesions, and antioxidant enzymes (PPO and POD) and total soluble protein contents increased (Farooq et al. 2021). The chemistry behind the interaction between nanomaterials and proteins has been explained by Zhang et al. (2018).

9.8 Interaction of NPs with Hormones

The original definition of plant hormones can be stated as naturally occurring compounds that showcased physiological activity within plants (Weyers and Paterson 2001). Physiological activity is in reference to growth, maturation and towards stress responses in a plant. Some may cause the elongation of roots or shoots while others may be involved in senescence (Yang et al. 2017). The plant hormones are classified based upon their chemical structure and/or their physiological activity (Weyers and Paterson 2001). Thus plant hormones have been classified into five main classes of naturally occurring plant hormones: auxin, cytokinin, gibberellins, abscisic acid and ethylene (Gaspar et al. 1996). This system of classification thus giving "classical five" came into existence only since 1937 before which under the category of phytohormones, only auxins existed (Kende and Zeevaart 1997). Apart from the classical five, there are plant hormones that exist such as brassinosteroids, salicylic acid, jasmonic acid and strigolactones. Strigolactones have been discovered to contribute towards the plant growth and development as well as respond to biotic and abiotic stresses (Bari and Jones 2008).

Hormonal routes were examined in Bright Yellow 2 (BY-2) tobacco (*Nicotiana tabacum* L.) cell suspensions treated with manganese ferrite nanoparticles ($nMnFe_2O_4$) or nitrogen-doped carbon dots (N-CDs). N-CDs (2.9 ± 0.5 nm) at 0.1 mg/L and $nMnFe_2O_4$ (28.7 ± 7.7 nm) at 1 mg/L from the dosage response study significantly increased cell survival by 11.6% and 30.4%, respectively. The cell shape, hormone content, H^+, K^+, and IAA fluxes, gene expression, and metabolite profiles were assessed for a subset of samples based on this information. Fresh weight and cytokinin levels were respectively increased by 48.2% and 70.22% after exposure to $nMnFe_2O_4$ and by 30.82% and 37.22%, respectively, after exposure to N-CDs. Specifically, $nMnFe_2O_4$ enhanced the expression of expansin by 3.3 fold for cell expansion by increasing the outflow of H^+ (by 75.7%) and influx of K^+ (by 278%). N-CDs caused late extrusion of H^+ and early expression of CYCB (by 4.9 fold) for the cell cycle. Metabolomic research also showed that N-CDs contributed to stress resistance (by 41.8–121%) and IAA synthesis, while $nMnFe_2O_4$ increased metabolism in cytokinin signalling by 5.4–17.0%, ATP synthesis by 22.6–200%, and cell growth (27.5%) by 63.3%. Together, it appears that $nMnFe_2O_4$ started the SCFTIR1/AFB-AUX/IAA signaling pathway, and N-CDs may have turned on the IAA-mediated cell cycle to encourage cell proliferation (Wang et al. 2022).

SeNPs synthesized by reducing H_2SeO_3 with ascorbic acid and arabic gum had spherical shape and an average size of 80 nm. When tested on gerbera in vitro, they performed well as a rooting agent and substituted for auxin. This led to improved phenotypic characteristics, such as 0.3 to 1.5 mg/L SeNPs decreased the rooting time, 0.7 to 1.5 mg/L increased leaf length, and 1 mg/L gave rise to the most chlorophyll (35.47 nmol/cm^2). 3 mg/L SeNPs led to the highest CAT activity (275.58 Umin^{-1} g^{-1}prot) and increased the ABA content to 4.33 times the positive control. 1.5 mg/L gave auxin content 6.14-fold higher, kinetin 2.78 higher and Gibberellic acid 4.70 higher than the negative control. 1 mg/L gave the highest SOD activity (43.13 Ug^{-1}prot ± 3.48). 0.7 mg/L SeNPs raised the zeatin levels to 4.79 times higher than the negative control. The highest plant survival (95%) was seen in 0.7, 1 and 1.5 mg/L SeNPs treatment, whereas plants treated with IBA and free-auxin showed lower survival (Khai et al. 2022).

The applications of NPs have been found in carrier systems for delivery systems to deliver such plant hormones. One such example is the delivery of the plant hormone gibberellic acid (GA_3) by nanocarriers such as alginate/ chitosan system or chitosan/ tripolyphosphate system. It was observed that both these nanocarriers were stable for 60 days but their release mechanism differed with temperature and pH. In addition, it was identified that when alginate/ chitosan system was used to deliver GA_3 in *Phaseolus vulgaris* L., there was an increase in leaf area as well as elevation in the levels of carotenoids and chlorophyll (Pereira et al. 2017). Yang et al. fabricated a core–shell structured made from a substrate of multiwalled nanotubes (MWNTs), which could be used to trace presence of phytohormones in fruit juices (Yang et al. 2022).

NPs have also been used to study phytotoxicity of plant hormones in plants when they surpass the adequate limit in terms of quantity required for their various activities (Weyers and Paterson 2001). NPs can increase in the plants defense hormones like salicylic acid, jasmonic acid, abscisic acid, zeatin riboside and brassinosteroids (Farooq et al. 2021). Silica NPs improve disease resistance via salicylic acid-mediated systemic acquired resistance (El-Shetehy et al. 2020).

Eleven biochemical pathways were altered in maize leaves due to the growth conditions of 100 mg Fe_3O_4NP/kg of soil, related to glycolysis and gluconeogenesis, like the TCA cycle (Zhao et al. 2019). This affects the synthesis of plant hormones like salicylic acid, ethylene, and auxin, as well as amino acids (Metch et al. 2018). The same concentrations of SiO_2, TiO_2 and Fe_3O_4 also increase 4-aminobutyric acid (GABA) and its precursor glutamic acid, which have many roles including signal transduction and stress defense (Shelp et al. 1999). GABA produces the stress response chemical succinate semialdehyde in the mitochondria, and this is increased upto eightfold due to the soil treatment (Allan et al. 2012). This indicates that the plant sensed the root stress and accordingly readied the shoot tissues. 4 pathways were disrupted in the roots: inositol phosphate (a secondary messenger for extracellular signals), ascorbate, aldarate, glycerolipid metabolism (related to antioxidant plant defense) and the TCA cycle. This shows that Fe_3O_4 causes stress response. TiO_2 additionally affected the methane production pathway.

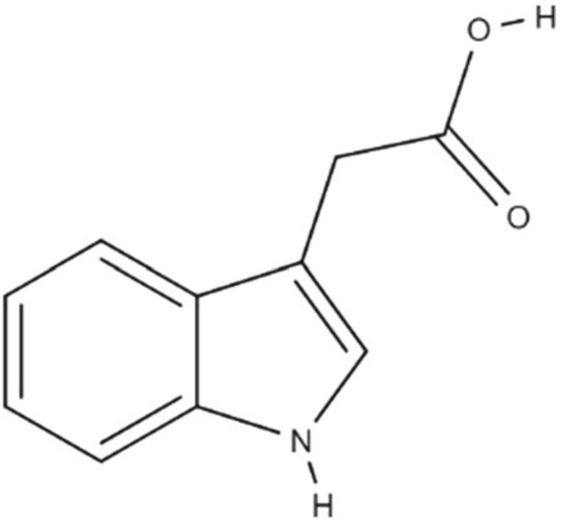

Fig. 9.5 Structure of IAA rendered on MolView

9.8.1 Effect on Levels of IAA

Indole-3-acetic acid (IAA) is the main naturally-occurring auxin (Fig. 9.5). This auxin is primarily produced in plants. This is achieved by a de novo biosynthesis pathway which utilizes tryptophan as a pivotal precursor. Through this pathway, the IAA generated is utilized in the processes of seedling growth, flower development, embryogenesis and vasculature (Zhao 2012). 25- to 100-mg/L GO exposure in rapeseed causes decrease in IAA levels (Cheng et al. 2016).

In the IAA transcription process of transgenic plants, IAA4 and IAA10 are downregulated, and IAA7 is upregulated. In the wild type, IAA7 is downregulated; IAA10 is upregulated (Jiao et al. 2016). However, it was observed that when CeO_2-NPs were used, at a concentration of 500 mg/L there was significant increase in the levels of IAA in the leaves of conventional cotton but not in the transgenic cotton leaves. In another study, the presence of carbon nanotubes in rice, IAA levels decreased in the shoot regions as well as in the root regions. When CuO at an intensity of 10 or 200 mg/L interacts with shoot tissue of lpt-cotton, IAA levels declined while at a concentration of 1000 mg/L, in the root tissues of the same plant, IAA levels increased (Yang et al. 2017).

9.8.2 Effect on the Levels of Abscisic Acid (ABA)

Abscisic acid is a sesquiterpenoid which implies that its structure comprises 15 carbon atoms (Fig. 9.6) (Rai et al. 2011). It has numerous functional moieties and a non-planar structure. ABA can be synthesized from mevalonate or from beta carotene and it is produced primarily under conditions when the plant is under some form of stress. This may be in the form of drought, radiation, heavy metal, heat or thermal stress. On the whole, ABA is active during the time when the plant experiences abiotic stress. ABA responds by acting on and thus modifying the expression level of genes especially the *cis*- and *trans*- acting regulatory elements of responsive promoters (Vishwakarma et al. 2017). Apart from stressed conditions, ABA is crucial for the control of numerous plant physiological systems. For instance, in plant tissue culture systems, ABA is used to promote somatic embryogenesis. The presence of ABA enhances the quality of the somatic embryos by preventing precocious germination and increasing desiccation tolerance (Rai et al. 2011).

Concentrations of 25- to 100-mg/L GO exposure in rapeseed cause abscisic acid (ABA) levels to increase (Cheng et al. 2016). In tomato, ABA limiting enzyme NCED transcription with exposure to 20 mg/L GO, especially in transgenic plants, indicates that the rd29A activator (drought and salt resistance factors) is activated by GO; highly transcribed genes include ABCG25 and ABCG40. In the wild type, ABCG25 and ABCG40 are not affected (Jiao et al. 2016). When CeO_2 at a 100 mg/L concentration was present in Bt-transgenic cotton, the levels of ABA in the root tissue decreased. Furthermore, the presence of Fe_2O_3 in peanuts at a concentration

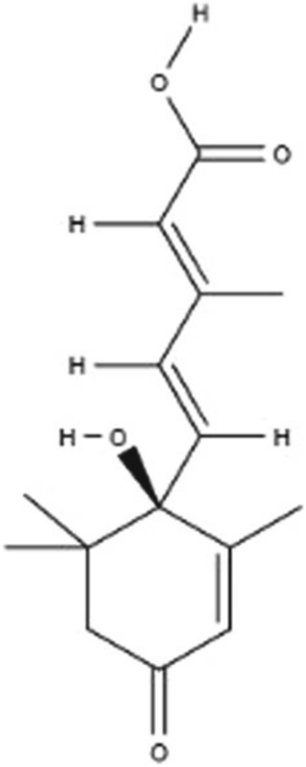

Fig. 9.6 Structure of ABA rendered on MolView

of 250 mg/L decreased the ABA content in tissues of the shoot while a concentration of 1000 mg/L decreased the ABA content in the root tissues. Another interesting finding is that in lpt-cotton, when the amount of CuO NPs was around 10 mg/L, the concentration of ABA decreased, but when it was around 200 or 1000 mg/L, the concentration of ABA increased in both the root and shoot tissues (Yang et al. 2017).

Treatment of *Arabidopsis* wild-type and mutant line gl-1 (glabra-1) plants that lack trichomes on stems and leaves with silver NPs showed a change in stress-related phytohormones and their derivatives. A 6 h treatment of 150 mg/L AgNPs boosted jasmonic acid, abscisic acid and salicylic acid levels. Abscisic acid and its metabolites phaseic acid (PA) and dihydrophaseic acid (DPA) showed an increase in the wild type by 7.9-fold and in gl-1 by 16.9-fold as compared through treatment with silver ions. gl-1 line showed a 1.4-fold increase of jasmonic acid and its precursor (cis-(+)-12-oxo-phytodienoic acid) by 2.5-fold, and hydroxy-JA catabolites by 2.3 times, compared with the wild type (Angelini et al. 2022).

9.8.3 Interaction with Gibberellins

Gibberellic acid is used to activate enzymes, stimulate dormant seeds, and encourage plant growth by increasing the rate of cell division. It can also stimulate fruit growth, prevent senescence and increase yields (Quamruzzaman et al. 2021). Consequently, the appropriate use of GA is important for agriculture. Like the above-mentioned alginate/ chitosan system (Fig. 9.7) or chitosan/ tripolyphosphate systems, engineered NPs are being used for precision delivery of growth hormones to the plant tissues. This ensures less wastage of chemicals and a controlled release of the hormone. For instance, mesoporous silica NPs were used to store salicylic acid, and a decanethiol gatekeeper system caused the hormone to be released when glutathione was present (Yi et al. 2015).

Hollow mesoporous nanocarriers of silica with installed Fe_3O_4NPs functionalized with water soluble carboxylatopillar [5]arene ammonium are used as nanovalves. The structures are loaded with gibberellin GA_3, and the host–guest interactions are bidirectional and responsive to changes in environment. It was tested in *A. thaliana* L. and cabbages. The hormones were released in conditions of pH over 5 or pH less than 4, to help the plant respond to alkaline or acidic soil or water stress. The iron portion makes them magnetic for an extra layer of control. They could also respond to 1,4-butanediamine (BDA) and ultrasound (Li et al. 2019).

Another gibberellin delivery system used a porous metal–organic frameworks (MOFs) with a nanovalve made of carboxylated leaning tower[6]arene (CLT6) in Chinese cabbage and wheat. It was functionalized by coordination with quaternary ammonium stalks, which gave it a diameter of 101 nm and a zeta potential value of −13.2 mV (Yang et al. 2021). Gibberellin delivered this way increased the seed germination of the two species by 1.86 and 1.30-fold respectively. Leaning tower[6]arene is an improvement over Pillar[n]arenes due to higher yield during synthesis, cavity adaptability, hormone binding capacity, and fewer substituents. The

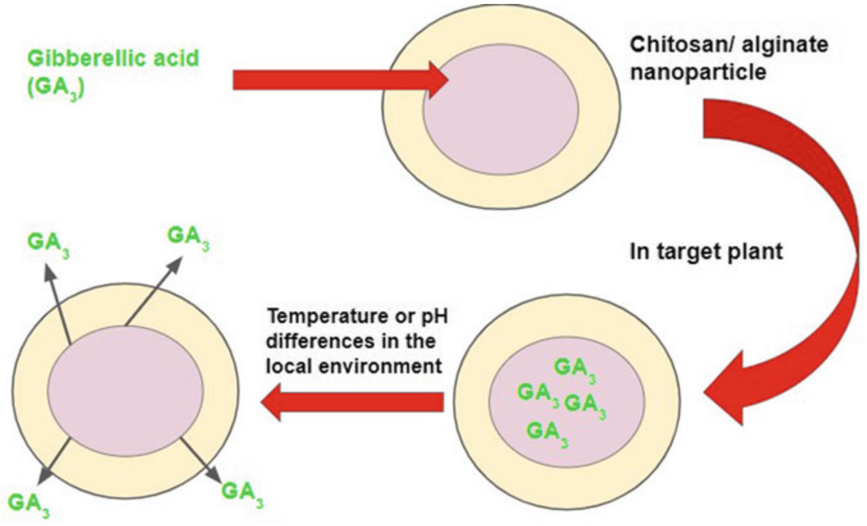

Fig. 9.7 Effect of Chitosan/Alginate NPs on phytohormones

hormone cargo was released at lower pH of 5–6 due to reduced host–guest interaction of the anionic CLT6 macrocycles and cationic Q stalks. Higher temperature caused release due to decreased complex stability. Release was also mediated through the strong competitive binding of polyamines, chemicals common to plant tissues. The study by Yang et al. (2021) explains how loading capacity and encapsulation efficiency can be calculated. These innovations are paving the path towards modern precision agriculture.

9.9 Conclusion and Prospects

NPs are structures which are in the size range of 1–100 nm. They are important due to the fact that owing to their size, physical and chemical properties, they can be used in a variety of systems to deliver a compound or simply to be used in toxicity studies of a system. Regardless, in all these conditions, the NPs interact with the system which can be a plant wherein the interaction occurs with macromolecules such as nucleic acids, proteins and hormones. The interactions observed are usually electrostatic, π-π, covalent and Van der Waals in nature. However, the type of interaction differs with the type of NPs. The interactions of metal oxide NPs of zinc, copper, magnesium, iron, titanium, silica, cadmium sulphide and quantum dots have been explored. Graphene NPs include graphene oxide and different carbon nanotubes (CWTs). Here modifying the surface chemistry changes the properties of the NPs. Then, there are arabinoxylan NPs which have been prepared from wheat bran waste and chitosan NPs which have been used to deliver gibberellic acid in certain plant systems. BioClay NPs have also been used to deliver DNA and RNA by binding interactions. The effect of these NPs can be observed as they can modulate gene transcription based upon the NP being used. In addition, these particles can affect the growth of the plant and can induce a stress condition which causes an effect on the SOD, POD, and CAT, among other enzymes, are expressed and active. With regards to applications, nanomaterials have been used as sensors for food allergens, toxicity testing and to protect the plant from virus infection. More futuristic devices for highly individualized crop plant sensing take the form of high-tech nanodevices like a graphene-based wearable sensor developed by Oren et al. which monitors water evaporation from plant leaves (Oren et al. 2017). It senses this due to variations in the electrical resistance of graphene under various humidity conditions. Pt nano sensors functionalized with IgG antibodies were used to find bacteria associated with plants in soil and carrots (Ahmad et al. 2012). By understanding the interactions of a plant in its environment, NPs that mimic these relationships could go a long way in saving resources through precision pesticides, fertilizers and stress detectors. These can be applicable for both the agricultural setting and for monitoring endangered species, or even invasive ones without harming the native plants. More interdisciplinary studies for the intelligent design and deployment of these exemplary machines are required, especially to assess their safety in advance and to search for better materials that can be created through green synthesis and can breakdown once their purpose is served. Thus the future prospects of nanomaterial's coexistence with plants are bright and further research can only uncover more efficient systems.

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Chapter 10
Influence of Nanomaterials on Non-enzymatic Antioxidant Defense Activities in Plants

Antonio Juárez-Maldonado

Abstract Plants are exposed to different types of stress throughout their development. In order to tolerate these stressful conditions, plants have a series of systems that allow them to defend themselves and successfully complete their life cycle. One of the most important is the antioxidant defense system, through which enzymatic and non-enzymatic antioxidant compounds are synthesized. Antioxidants are necessary as they prevent reactive oxygen or nitrogen species produced at the cellular level from damaging different cellular structures and consequently the integrity of the entire plant. In particular, non-enzymatic antioxidants have the great advantage of being non-specific which is why they can neutralize a variety of reactive species. Therefore, it is necessary to maintain adequate antioxidant production to avoid any type of damage caused by reactive species. Nanomaterials have proven to be an effective tool to induce positive responses in plants. This is due to the unique characteristics they possess due to their size ranging from 1 to 100 nm. Nanomaterials can be applied to plants by different routes and enter cells. In this journey, they interact with the different cell structures such as cell wall, cell membrane, different organelles and even with the nucleus, this induces a series of responses that range from the production of reactive oxygen species, to the modification of different metabolic pathways and also gene expression. Finally, the result is the increase in the production of different secondary metabolites and antioxidants such as phenols, flavonoids, alkaloids, carotenoids, ascorbic acid, etc. Adequately knowing the mechanism of action of nanomaterials on the antioxidant system of plants can be useful to plan management strategies aimed at obtaining specific results such as the increase in non-enzymatic antioxidants that are useful not only for the plant but also for human health.

Keywords Biostimulation · Gene expression · Metabolic pathways · Nanotechnology · Reactive oxygen species · Secondary metabolism · Stress

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10.1 Introduction

Plant species are organisms that in most of their development are exposed to different environmental conditions that are outside their optimal level. These conditions, commonly called stress, can be biotic or abiotic in origin (Ramegowda and Senthil-Kumar 2015). Global warming and climate change have made these stressful conditions increasingly common. As a result, the productivity of plants can be affected to a greater extent. To defend themselves against different stress conditions, plants have a series of mechanisms and systems that allow them to tolerate stress and thus be able to complete their life cycle. One of the most important systems is the antioxidant defense system that involves the production of enzymatic and non-enzymatic antioxidants (Choudhary et al. 2020). Antioxidants have the ability to neutralize reactive oxygen species (ROS) or reactive nitrogen species (RNS), which are always produced at the cellular level under a stress condition; their overproduction causing oxidative stress (Bhattacharjee 2012). This can generate different types of damage at the cellular level such as cytotoxicity, genotoxicity, lipid peroxidation, apoptosis, intracellular protein degradation, and miRNA dysregulation (Rahmani et al. 2020).

Non-enzymatic antioxidants are of utmost importance in plant metabolism. Since they have several advantages over enzymatic ones, unlike antioxidant enzymes, are not specific type. Therefore, they can neutralize a variety of ROS and RNS (Haida and Hakiman 2019). In addition, non-enzymatic antioxidants participate in other metabolic functions that are vital for plants (Rajput et al. 2021), e.g., carotenoids can dissipate excessive energy or even function as accessory pigments (Edge and Truscott 2018). In addition, phenols and flavonoids perform a variety of functions in plants, commonly related to tolerating different stress conditions (Vasconsuelo and Boland 2007; Thakur and Sohal 2013). Additionally, non-enzymatic antioxidants are important for human health, as they have beneficial effects on different non-communicable diseases (Saini et al. 2015; Cisternas-Jamet et al. 2020; Cortés-Estrada et al. 2020).

Nanotechnology through the use of nanomaterials (NMs) (materials on a scale of 1–100 nm) has been an extremely useful tool in the development of science. This has been achieved due to the unique properties of NMs that can be exploited for a variety of applications in different areas of scientific applications (Juárez-Maldonado et al. 2019; Bai et al. 2021). Particularly, the application of NMs in plant species has been shown to have enormous beneficial effects, ranging from greater plant growth and development, to increasing the ability to tolerate both biotic and abiotic stress conditions (Rizwan et al. 2017; Kumar et al. 2019; Awasthi et al. 2020).

One of the main impacts of the NMs application in plants is the modification of the antioxidant defense system, which stimulate the production of enzymatic and non-enzymatic antioxidants (Lala 2021; Juárez-Maldonado et al. 2021). This is accomplished due to the ability to modify cell metabolism and gene expression (Khodakovskaya et al. 2012; Yan et al. 2013). This chapter describes the mechanism through which NMs impact the production of non-enzymatic antioxidant compounds in plants. In addition, the influence of the different NMs applied through different routes on the production of non-enzymatic antioxidants in plants is reviewed.

10.2 Antioxidant Defense System in Plants

The antioxidant defense system of plants controls reactive oxygen species, since a balance is required between the production of ROS and their neutralization due to the effect of antioxidants. If this antioxidant defense system did not exist, the overproduction of ROS would cause oxidative stress at the cellular level and damage to the different cellular structures. The antioxidant defense system includes the production of enzymatic and non-enzymatic compounds that will neutralize ROS, thus preventing damage (Choudhary et al. 2020). However, at low concentrations, ROS have the ability to function as signaling molecules and stimulate the production of compounds such as abscisic acid, salicylic acid, or jasmonic acid, that can induce tolerance to different stress conditions (Mertens et al. 2021). Thereby, ROS can be considered as compounds with double functions. Their high concentrations will cause oxidative stress and at low concentrations stimulate the production of beneficial compounds in plants.

10.2.1 Plant Stress

Plants, being living organisms, unable to move from one place to another. They are obliged to develop their entire life cycle in the same place and they will be exposed to different stress conditions including abiotic and biotic during developmental life cycle (Ramegowda and Senthil-Kumar 2015). Stress is not an optimal condition, unsuitable for the plant growth and development, prevents the maximum expression of their growth and yield potential (Shao et al. 2008). Therefore, any stress condition regardless of its origin (biotic or abiotic) will result in the reduction of the total plant productivity. The difference between the optimal and stressful conditions in the total plant productivity is known as the loss due to the stress factor (Atkinson and Urwin 2012; Rejeb et al. 2014).

Each stressor induces different type of response in plants, e.g. drought inhibits photosynthesis and increases abscisic acid (ABA), but also induces the production of ROS that generate oxidative stress (Ghosh and Xu 2014). High temperature stress decreases photosynthesis, induces membrane damage, affects carbon and nitrogen metabolism, and also induces oxidative stress (Asseng et al. 2011; Dias et al. 2011). Salt stress also negatively affects photosynthesis, induces osmotic stress, ionic toxicity, and also oxidative stress (Hanin et al. 2016; Zörb et al. 2019). Any type of abiotic stress induces ROS overproduction and exerts oxidative stress.

In plants, biotic stress is caused by living organisms (fungi, bacteria, oomycetes, viruses, nematodes, insects and weeds) (Saddique et al. 2018; Araújo et al. 2020;

Esgario et al. 2020). Under biotic stress conditions, the changes occur at biochemical, morphological, molecular aspects in plants (Zaynab et al. 2019). Plants recognize pathogens through transmembrane pattern recognition receptors (PRR), which is a part of the defense pathway called pathogen-associated molecular pattern (PAMP) activated immunity (PTI) (Monaghan and Zipfel 2012). There is also a second pathway called effector-activated immunity (ETI) where intracellular resistance protein receptors directly recognize specific pathogenic effectors or indirectly their interaction effects on host cell components (Sang et al. 2020; Yoo et al. 2020). While in the case of herbivorous organisms, plants detect them through damage-associated molecular patterns (DAMP) (Quintana-Rodriguez et al. 2018), or by herbivore-associated molecular patterns (HAMP) (Si et al. 2020).

In any case, the different biotic stress detection pathways trigger a series of responses such as ROS generation, calcium ion signaling, production of proteins related to pathogenesis (PR), changes in levels of salicylic acid (SA) and jasmonic acid (JA) (Monaghan and Zipfel 2012; Quintana-Rodriguez et al. 2018; Yoo et al. 2020; Si et al. 2020). Basically, any stress condition, ultimately induces an overproduction of ROS and consequently induces oxidative stress.

10.2.2 Reactive Oxygen Species

Photosynthesis is the physiological process through which life itself develops, since from this process sugars are synthesized that serve as the basis for the metabolism of plants. Photosynthesis uses carbon dioxide (CO_2) from the atmosphere and releases molecular oxygen (O_2). The activation or reduction of oxygen leads to the production of reactive oxygen species (ROS), among which are singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and others as perhydroxyl radical ($HO_2^{\bullet -}$), alkoxy radical ($RO^\bullet$), peroxy radical ($ROO^\bullet$), and organic hydroperoxide (ROOH) (Bhattacharjee 2012; Tripathy and Oelmüller 2012; Rajput et al. 2021). One of the main ROS is the superoxide anion ($O_2^{\bullet -}$), which can be switched to form hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^\bullet$) (Gutowski and Kowalczyk 2013).

ROS are produced both in plants and in other living organisms in chloroplasts, mitochondria and peroxisomes mainly, due to the processes of photosynthesis and respiration (Tripathy and Oelmüller 2012; Kundu et al. 2018). Furthermore, ROS can be produced in the cell wall due to the presence of peroxidases and amine oxidases, and in the plasma membrane due to the effect of NADPH oxidase, as well as in the cytosol, glyoxysomes and endoplasmic reticulum (Tripathy and Oelmüller 2012; Janků et al. 2019).

Although it is true that ROS are part of the aerobic metabolism of plants, their overproduction is risky, since it can induce what is known as oxidative stress (Bhattacharjee 2012). Oxidative stress is the increase in oxidizing agents with the ability to extract electrons from organic molecules and therefore disrupt their cellular function. The excessive production of ROS can induce oxidative damage in different cell organelles by affecting proteins, lipids and even DNA (Tripathy and Oelmüller 2012). However, plants can regulate the accumulation of ROS in such a way as to avoid oxidative damage at the cellular level. For this, plants take advantage of their antioxidant defense system, which is composed of a series of enzymes (superoxide dismutase [SOD], catalase [CAT], ascorbate peroxidase [APX]) that allow the elimination of ROS, as well as electron donor compounds called non-enzymatic antioxidants (ascorbic acid, glutathione, phenolic acids, alkaloids, flavonoids, carotenoids, etc.), thus limiting the possible harmful effects of ROS (Hasanuzzaman et al. 2020).

10.2.3 Enzymatic and Non-enzymatic Antioxidants

Plants commonly keep their antioxidant defense system active to maintain redox homeostasis (ROS production/ROS scavenging) (Miller et al. 2010). The compounds used in this system to avoid oxidative stress are called antioxidants, which are compounds that work as electron donors to neutralize free radicals (Haida and Hakiman 2019).

Antioxidants can be of the enzymatic type such as superoxide dismutase, catalase, ascorbate peroxidase or of the non-enzymatic type such as ascorbic acid, glutathione, phenolic acids, alkaloids, flavonoids and carotenoids (Kapoor et al. 2019; Hasanuzzaman et al. 2020). This classification into enzymatic or non-enzymatic compounds depends on their catalytic action, where enzymatic antioxidants require cofactors and are highly specific for substrates, while non-enzymatic antioxidants do not have any specific substrate (Haida and Hakiman 2019). These antioxidants work as a defense against the damage that ROS can cause and therefore to maintain the health of plant cells, in addition to the fact that antioxidants are also important because they participate in a series of functions and mechanisms that influence development of the plants (Rajput et al. 2021).

SOD dissolves $O_2^{\bullet-}$ to O_2 and H_2O_2, while the enzymes peroxidases (POX), CAT, and GPX work on H_2O_2 and HO_2 to convert them into water and (O_2), or lipid alcohols (Rajput et al. 2021). These enzymes can perform their function thanks to other non-enzymatic compounds that function as electron donors. GPX by reducing H_2O_2 and HO_2 to water and lipid alcohols, uses thioredoxin as an electron donor (Rajput et al. 2021). While APX uses ascorbate as specific electron donor to convert H_2O_2 to water (Pandey et al. 2017).

Non-enzymatic antioxidants have the great advantage that, as they are not specific to any substrate, they can neutralize both ROS and RNS to different free radicals (Haida and Hakiman 2019). Antioxidants can also be classified according to their solubility as soluble in water (hydrophilic) or soluble in fat (lipophilic) (Kumar et al. 2017; Haida and Hakiman 2019). However, in plants most of the antioxidants are hydrophilic, among which are phenols, flavonoids, SOD, CAT, GPX, as well as uric, lipoic, benzoic, and ascorbic acids. Lipophilic antioxidants include carotenoids, tocopherols, vitamin K, ubiquinone, and phospholipids (Haida and

Hakiman 2019). The ascorbic acid and glutathione, phenolic acids, flavonoids, alkaloids, and carotenoids are the main non-enzymatic antioxidants in plants (Gill and Tuteja 2010; Hasanuzzaman et al. 2020).

10.2.4 Stress and Antioxidant System

The ability of plants to neutralize ROS is of utmost importance since it directly influences their ability to tolerate different types of biotic or abiotic stress (Rajput et al. 2021). Although ROS can be toxic and damage different cell structures, they also function as signaling molecules that allow acclimatization to some type of stress (Choudhury et al. 2017). Virtually all types of abiotic stress such as salinity, extreme temperature, high light, and pollutants, induce increased ROS production (Kerchev et al. 2020). Hence, controlling or maintaining ROS levels at a point where they do not generate oxidative damage at the cellular level will help to tolerate different stress conditions.

10.3 Nanotechnology and Nanomaterials

Technology is a fundamental tool for the development of agriculture, since it allows improving agricultural systems enormously. One of the technologies that are currently available is that which is responsible for the study of materials on a scale of 1–100 nm and is known as nanotechnology. On this scale, materials have different and special characteristics that are very useful for different applications. NMs have a higher surface/volume ratio, which gives them a greater amount of surface free energy and therefore greater reactivity (Juárez-Maldonado et al. 2019), in addition to other properties such as shape, size, chemical composition, concentration, surface structure, aggregation and solubility (Bai et al. 2021).

At present there are different types of NMs such as metalloids and metal oxides, chitosan, or carbon-based nanomaterials as carbon nanotubes (CNT), graphene and fullerene, which have been used for different purposes in agriculture (Adeel et al. 2021; Ors et al. 2021; Seleiman et al. 2021).

NMs can induce biostimulation and favorable responses in plant growth and development, as well as positive changes in metabolism that can result in a greater ability to tolerate different stress conditions. This is due to the fact that NMs, when applied to plants, interact with cell walls and membranes, triggering a series of responses such as changes in the transport of metabolites and ions, or cell organelles already within the cell and can induce oxidative stress (Juárez-Maldonado et al. 2019, 2021). Ultimately the different types of interaction with plant cells translate into changes in metabolism that can be positive or negative, or even in some cases there are no responses (Juárez-Maldonado et al. 2021).

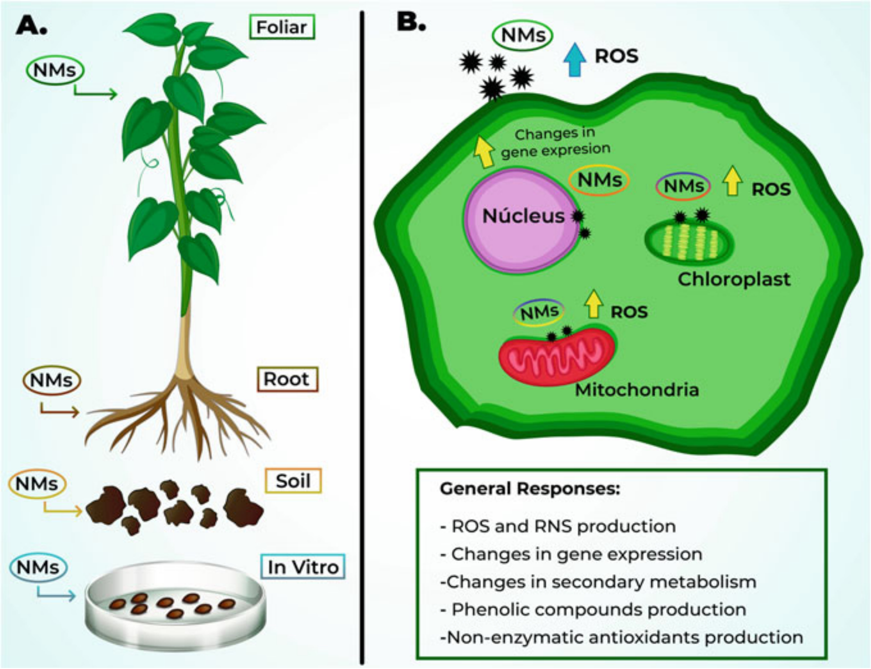
The stimulation of growth and productivity with the use of NMs is one of the main responses in plants, but an increase in the synthesis of antioxidant compounds, secondary metabolites such as phenols and flavonoids, among others, can also be observed (Rizwan et al. 2017; Kumar et al. 2019; Awasthi et al. 2020). However, these responses are dependent on the concentration of NMs applied, the type and size, and even on the plant species in which they are applied (Juárez-Maldonado et al. 2019, 2021).

In addition to being biostimulants, NMs are elicitors of secondary metabolites in plants and of bioactive compounds (Lala 2021; Rivero-Montejo et al. 2021). Also by inducing oxidative stress at the cellular level, NMs can activate the antioxidant defense system and therefore induce the production of enzymatic and non-enzymatic antioxidant compounds (Lala 2021; Juárez-Maldonado et al. 2021). Hence, the application of NMs in adequate amounts will induce a series of positive responses in plants, ranging from greater growth and development, synthesis of compounds or secondary metabolites, as well as the production and accumulation of antioxidants (Juárez-Maldonado et al. 2019, 2021).

10.3.1 Nanomaterials and Non-enzymatic Antioxidant Defense

Due to the high surface energy and a high surface/volume ratio, NMs present a greater reactivity and biochemical activity that may impact plants in various ways (Juárez-Maldonado et al. 2019). One of the main impacts is the induction of ROS and as a consequence an oxidative stress occurs at the cellular level (Marslin et al. 2017), this in turn activates the antioxidant defense system and the production of antioxidant compounds (Khan et al. 2017; Czarnocka and Karpiński 2018). At the cellular level, the interaction of NMs with cellular organelles induces oxidative stress (Juárez-Maldonado et al. 2021) due to the production of ROS and/or RNS that can cause cytotoxicity, genotoxicity, lipid peroxidation, apoptosis, intracellular protein degradation, and miRNA dysregulation (Rahmani et al. 2020). Furthermore, ROS function directly as signaling molecules that can modify secondary metabolism and therefore induce the production of different secondary metabolites such as phenols, flavonoids, alkaloids, etc. (Marslin et al. 2017; Silva et al. 2020) (Fig. 10.1).

But also NMs can directly interact with DNA and modify the expression of various genes that participate in different metabolic pathways (Khodakovskaya et al. 2012; Yan et al. 2013), and therefore, increase the production of various compounds among which are non-enzymatic antioxidants. There is evidence that the application of Fe NPs in *Zea mays* L. positively modifies some important metabolic pathways linked to the synthesis of non-enzymatic antioxidants such as ascorbic acid, phenols and flavonoids. Among the main modifications due to the application of Fe NPs, it was observed that the ascorbate and aldarate metabolism and the tricarboxylic acid cycle were activated (Li et al. 2020). In wheat plants (*Triticum aestivum* L.) the application



General Responses:

- ROS and RNS production
- Changes in gene expression
- Changes in secondary metabolism
- Phenolic compounds production
- Non-enzymatic antioxidants production

Fig. 10.1 **A** NMs can be applied to plants through different routes, either under in vitro conditions, directly to the soil or substrate, to the root, or foliar way. **B** Once they enter the plant, they interact with cell walls and membranes, triggering a series of responses such as the production of ROS. Upon entering the cell, they interact with the different cellular organelles inducing ROS, changes in gene expression and secondary metabolism that result in the production of phenolic compounds and diverse antioxidant compounds (Figure constructed by Yolanda González-García)

of TiO_2 NPs up-regulated the metabolisms of reserve sugars and tocopherol, and the phenylalanine and tryptophan pathways, the aspartate family pathway together with serine, alanine and valine metabolisms and the glycerolipids biosynthesis (Silva et al. 2020).

Another important route that can be modified is the plastidic isoprenoid biosynthetic pathway, which is where carotenoids are synthesized, which are very important compounds that participate as accessory pigments in chloroplasts, energy dissipators and antioxidants (Joyard et al. 2009). There is evidence that the application of various NMs can induce the accumulation of carotenoids in different plant species such as Al_2O_3 NPs in *Hibiscus sabdariffa* L. (Abdel Latef et al. 2020), CeO_2 NPs in *Dracocephalum moldavica* L. (Mohammadi et al. 2021), chitosan nanoparticles (CS NPs) in *Vigna radiata* L. (Sen et al. 2020), Fe_2O_3 NPs in *Lactuca sativa* L. (Jurkow et al. 2020), even multilayer carbon nanotubes (MWCNT) in *Catharanthus roseus* L. (Ghasempour et al. 2019), among other (See Table 10.1).

In any case, the application of NMs in plants will induce a series of responses linked to the antioxidant defense system and secondary metabolism that will result

Table 10.1 Induction of non-enzymatic antioxidants in different plant species with the application of nanomaterials

Plant specie	Increased Antioxidant	Nanomaterial	Concentration and route of application	Reference
Allium cepa L.	Phenols	Ag NPs	10, 15, 25 and 50 μg/mL/Nutritive solution	Casillas-Figueroa et al. (2020)
Allium cepa L.	Terpenoids	Ag NPs	40 and 75 mg/L/Foliar	Fouda et al. (2020)
Lactuca sativa L.	Ascorbic acid	Ag NPs	10 and 50 mg/L/Foliar	Wu et al. (2020)
Pennisetum glaucum (L.) R.Br	Phenols, flavonoids	Ag NPs	10, 20 and 30 mM/seed priming	Khan et al. (2020)
Momordica charantia L.	Phenols, flavonoids, tocopherols	Ag NPs	1, 5 and 10 mg/L/Added to the MS culture medium	Chung et al. (2018)
Caralluma tuberculate N.E.Br	Phenols, flavonoids	Ag NPs	30, 60 and 90 μg/L/Added to the MS culture medium	Ali et al. (2019a)
Vigna radiata L.	Phenols, alkaloids	$AgNO_3$ NPs	20, 50, 100 mg/L/Seed priming	Kumari et al. (2017)
Nigella arvensis L.	Alkaloids, flavonoids	Al_2O_3 and NiO NPs	100 and 1000 mg/L/Hydroponic solution	Modarresi et al. (2020)
Hibiscus sabdariffa L.	Carotenoids	Al_2O_3 NPs	0.01%/Seed priming	Abdel Latef et al. (2020)
Dracocephalum moldavica L.	Carotenoids	CeO_2 NPs	25, 50 and 100 mg/L/Foliar	Mohammadi et al. (2021)
Hordeum vulgare L.	Carotenoids	$CoNd_2Fe_{1.8}O_4$ NPs	500 and 1000 mg/L/Added to seeds in filter paper	Tombuloglu et al. (2020)
Catharanthus roseus (L.) G.Don	Alkaloids	CS NPs	5 mg m/L/Foliar	Hassan et al. (2021)
Capsicum annuum L.	Phenols	CS NPs	1%/Foliar	Taheri et al. (2020)
Oryza sativa L.	Phenols	CS NPs	2 mg/mL/Foliar	Divya et al. (2020)

(continued)

Table 10.1 (continued)

Plant specie	Increased Antioxidant	Nanomaterial	Concentration and route of application	Reference
Camellia sinensis (L.) Kuntze	Gallic acid, epicatechin, epigallocatechin gallate, gallocatechin gallate, epicatechin gallate	CS NPs	0.001%/Nutritive solution	Chandra et al. (2015)
Vigna radiata L.	Carotenoids, phenols, flavonoids, ascorbic acid	CS NPs	Seed priming	Sen et al. (2020)
Capsicum annuum L.	Phenols, flavonoids	Cu NPs	100 and 500 mg/L/Foliar	González-García et al. (2021)
Annona muricata L.	Flavonoids	Cu NPs	250, 500, 750 and 1000 mg/L/Foliar	Jonapá-Hernández et al. (2020)
Triticum aestivum L.	Phenols	Cu NPs	25 and 50 mg/kg/soil	Noman et al. (2020)
Capsicum annuum L.	Ascorbic acid	Cu NPs	500 mg/kg/Soil	Rawat et al. (2019)
Lactuca sativa L.	Flavonoids	CuO NPs	100 and 1000 mg/L/Foliar	Xiong et al. (2021)
Nicotiana tabacum L. cv. Virginia	Flavonoids	CuO NPs	25 mg/L/Added to MS culture medium	Mahjouri et al. (2018)
Gymnema sylvestre R.Br	Phenols, flavonoids	CuO NPs	3 mg/L/Added to MS culture medium	Chung et al. (2019a)
Dracocephalum moldavica L.	Phenols, flavonoids	Fe_2O_3 NPs	30, 60 and 90 mg/L/Foliar	Moradbeygi et al. (2020)
Lactuca sativa L. var. *Foliosa Bremer*	Carotenoids, phenols, ascorbic acid	Fe_2O_3 NPs	0.75%/Foliar	Jurkow et al. (2020)
Melissa officinalis L.	Phenols	Fe_2O_3 NPs	5, 10, 20, 30 and 40 μM/Drench	Mohasseli et al. (2020)
Triticum aestivum L.	Carotenoids	Fe_2O_3 NPs	500 mg/L/Foliar	Al-Amri et al. (2020)

(continued)

Table 10.1 (continued)

Plant specie	Increased Antioxidant	Nanomaterial	Concentration and route of application	Reference
Triticum aestivum L.	Ascorbic acid	Fullerene C_{60}-OH_{20}	10, 40, 80, 120 nM/Seed priming	Shafiq et al. (2021)
Ocimum basilicum L.	Phenols Estragol, Methyl Chavicol, Germacreno D, Linalool	Graphene oxide NPs	50 and 100 mg/L/Foliar	Ganjavi et al. (2021)
Zea mays L.	Tocopherols, polyphenol, flavonoids	HgO NPs	100 mg/kg/substrate	Saleh et al. (2021)
Zea mays L.	Anthocyanins	Lignin NPs	80, 312 and 1250 mg/L/Seed priming	Del Buono et al. (2021)
Atropa belladonna L.	Phenols, flavonoids, alkaloids	Mn_2O_3 NPs	25, 50 and 100 mg/L/Added to MS culture medium	Tian et al. (2018)
Cucumis sativus L.	Phenols	MoS_2 and TiO_2 NPs	27 mg/L/Foliar	Song et al. (2020)
Hordeum vulgare L.	Carotenoids	$NiFe_2O_4$ NPs	125, 250, 500 and 1000 mg/L/Hydroponic solution	Tombuloglu et al. (2019)
Calendula officinalis L.	Reduced glutathione, ascorbic acid	MWCNT	50, 100, 250, 500, 1000 mg/L/Foliar	Sharifi et al. (2021)
Catharanthus roseus (L.) G.Don	Carotenoids, Phenols, Alkaloids	MWCNT	50, 100, 150 mg/L/Added to MS culture medium	Ghasempour et al. (2019)
Satureja khuzestanica L.	Phenols, flavonoids, rosmarinic acid, caffeic acid	MWCNT	25, 50, 100 and 250 µg/mL/Added to B5 culture medium	Ghorbanpour and Hadian (2015)
Thymus daenensis L.	Phenols, flavonoids	MWCNT	250 mg/L/Added to MS culture medium	Samadi et al. (2020)

(continued)

Table 10.1 (continued)

Plant specie	Increased Antioxidant	Nanomaterial	Concentration and route of application	Reference
Salvia nemorosa L.	Rosmarinic acid, salvianolic acid B, ferulic acid, cinnamic acid	MWCNT-COOH	25, 50, 75, 100 mg/L/Added to MS culture medium	Heydari et al. (2020)
Brassica rapa L. ssp. *Pekinensis* var. Seoul	Anthocyanins	$NiSO_4$ NPs	50, 250 and 500 mg/L/Added to seed in filter paper	Chung et al. (2019b)
Capsicum annuum L.	Phenols	Se NPs	0.5, 1, 10 and 30 mg/L/Added to MS culture medium	Sotoodehnia-Korani et al. (2020)
Camellia sinensis L.	Polyphenols, carotenoids	Se NPs	2.5, 5, 10 and 20 mg/L/Foliar	Li et al. (2021)
Punica granatum L. cv. Malase Saveh	Phenols	Se NPs	2 μM/Foliar	Zahedi et al. (2019b)
Fragaria x ananassa (Weston) Duchesne	Carotenoids	Se NPs	10 and 20 mg/L/Foliar	Zahedi et al. (2019a)
Zea mays L.	Phenols	Si NPs	10, 50 and 150 mg/L/Foliar	Wang et al. (2021)
Vicia faba L.	Phenols	Si NPs	1.5 and 3 mM/Foliar	Hasan et al. (2020)
Triticum aestivum L.	Carotenoids	Si NPs	300, 600, 900, 1200 mg/kg/soil	Ali et al. (2019b)
Oryza sativa L. cv. Los IR-64	Anthocyanins, phenols, flavonoids and glutathione	SiO_2 NPs	60 mg/L/Drench	Banerjee et al. (2021)
Lycopersicon esculentum Mill	Carotenoids	SiO_2 NPs	20 mg/L/Seed priming	Madany et al. (2020)
Astragalus fridae Rech.f	Carotenoids	SiO_2 NPs	5, 40 and 80 mg/L/Seed priming	Moghanloo et al. (2019)
Hyoscyamus reticulatus L.	Phenols, alkaloids	SiO_2 NPs	25, 50, 100, 200 mg/L/Added to MS culture medium	Hedayati et al. (2020)

(continued)

Table 10.1 (continued)

Plant specie	Increased Antioxidant	Nanomaterial	Concentration and route of application	Reference
Dracocephalum kotschyi L.	Phenols, rosmaric acid	SiO_2 NPs	100 mg/L/Added to MS culture medium	Nourozi et al. (2019)
Coriandrum sativum L.	Carotenoids	TiO_2 NPs	100 mg/L/Foliar	Hu et al. (2020)
Raphanus sativus L.	Phenols	TiO_2 NPs	1500 mg/L/Foliar	Tighe-Neira et al. (2020)
Linum usitatissimum L.	Carotenoids	TiO_2 NPs	500 mg/L/Foliar	Aghdam et al. (2016)
Solanum lycopersicum L.	Lycopene	TiO_2 NPs	100, 500 and 1000 mg/kg/Foliar	Raliya et al. (2015)
Brassica oleracea L. var italic	Phenols	ZnO NPs	50, 100, 200, 400, 800 and 1000 μg/L/seed priming	Awan et al. (2021)
Triticum aestivum L.	Carotenoids	ZnO NPs	5, 10, 15 and 20 mg/L/seed priming	Rai-Kalal and Jajoo (2021)
Pisum sativum L.	Carotenoids	$ZnFe_2O_4$ NPs	5 μM/Drench	Abdelhameed et al. (2021)
Solanum lycopersicum L.	Lycopene	ZnO NPs	20 mg/kg/Soil	Ahmed et al. (2021)
Glycine max L.	Ascorbic acid	ZnO NPs	50 and 100 mg/L/Foliar	Ahmad et al. (2020)
Brassica napus L.	Flavonoids	Zn NPs	15 mg/L/Foliar	Sohail et al. (2020)
Phaseolus vulgaris L. var. Frijol pinto	Cryptoxanthin	ZnO NPs	500, 1000 and 2000 mg/L/Foliar	Salehi et al. (2021)
Solanum lycopersicum L.	Ascorbic acid	ZnO NPs	300 and 600 mg/kg/Soil	Akanbi-Gada et al. (2019)
Stevia reboudiana Bertoni	Phenols, flavonoids	ZnO NPs	0.1 and 1 mg/L/Added to MS culture medium	Javed et al. (2017)
Lupinus termis L.	Phenols, ascorbic acid	ZnO NPs	20 mg/L/Seed priming	Abdel Latef et al. (2017)

(continued)

Table 10.1 (continued)

Plant specie	Increased Antioxidant	Nanomaterial	Concentration and route of application	Reference
Arabidopsis thaliana (L.), Heynh	Carotenoids	ZnO NPs	300 mg/L/Drench	Wang et al. (2016)

in the production of non-enzymatic antioxidants such as glutathione, ascorbic acid, carotenoids, phenols and flavonoids, among others. In addition, the effect of NMs on the antioxidant system of plants is considered one of the main functions that are related to tolerance to different stress conditions (Mahto et al. 2021).

Several studies have demonstrated the impact of the application of NMs on the production of non-enzymatic antioxidant compounds through the modification of gene expression. In addition, it has been shown that the induction of these compounds occurs independently of the routes of application (Wang et al. 2021), found in corn (*Z. mays*) that the foliar application of Si NPs induces the expression of genes (*PAL*, *C4H*, *4CL*, *C3H* and *HCT*) linked to the biosynthesis of chlorogenic acid, which resulted in higher production of this compound and of all phenols in corn husks. Hassan et al. (2021) found that foliar application of chitosan nanoparticles in *C. roseus* increased the gene expression of mitogen-activated protein kinases (*MAPK3*), geissoschizine synthase (*GS*), and octadecanoid-derivative responsive AP2-domain (*ORCA3*), this resulted in a greater accumulation of alkaloids both in shoot and root.

The application of silicon dioxide nanoparticles (SiO_2 NPs) in the MS culture medium where *Dracocephalum kotschyi* L. was grew, induced an overexpression of phenylalanine ammonia-lyase (*PAL*) and rosmarinic acid synthase (*RAS*) genes related to rosmarinic acid (RA) biosynthetic pathway, and increased flavonoid content (xanthomicrol, cirsimaritin and isokaempferide) (Nourozi et al. 2019). While in *Hyoscyamus reticulatus* L. and *H. pusillus* the application of SiO_2 NPs directly to the root increased the expression of the genes *pmt* (putrescine N-methyltransferase) and *h6h* (hyoscyamine 6<beta>-hydroxylase), resulting in increased production of phenols and flavonoids, and accumulation of tropane alkaloid (hyoscyamine and scopolamine) (Hedayati et al. 2020).

The application of nickel oxide nanoparticles (NiO NPs) in the seeds of Chinese cabbage (*Brassica rapa* L. ssp. Pekinensis var. Seoul) induced an overexpression of the genes related to oxidative stress (*CAT*, *POD*, and *GST*), MYB transcription factors (*BrMYB28*, *BrMYB29*, *BrMYB34*, and *BrMYB51*), and phenolic compounds (*ANS*, *PAP1*, and *PAL*), this resulted in a higher content of anthocyanins, glucosinolates and phenolic compounds (Chung et al. 2019b).

10.4 Influences of NMs on Non-enzymatic Antioxidants

In the literature there is a vast amount of research that demonstrates the positive impact of the use of a variety of nanomaterials on the production of non-enzymatic antioxidant compounds in plants (Table 10.1). Among the most studied metallic or non-metallic NMs are NPs based on Ag, Al, Ce, Cu, Fe, Mn, Mo, Ni, Ti, Se, Si and Zn. In the case of carbon-based nanomaterials are graphene, fullerene and commonly MWCNT. In addition to these NMs, there are some others that have shown positive effects such as CS NPs that have been extensively studied recently, but also Lignin NPs. While the routes through which the NMs have been applied are foliar, seed priming, root using nutritive solution, soil or substrate, drench, or added to culture medium mainly.

10.4.1 Impact of NMs on Phenolic Compounds

Possibly one of the main effects of the application of NMs is the modification of the phenylpropanoid pathway, where phenolic compounds such as phenols, flavonoids, anthocyanins, and lignins, among others, are synthesized (Boudet 2007). In numerous plant species, the increase of phenols has been reported by the application of Ag NPs, such as in *Allium cepa* L., *Pennisetum glaucumK* L., *Momordica charantia* L., *Caralluma tuberculata* N.E.Br. (Chung et al. 2018; Ali et al. 2019a; Casillas-Figueroa et al. 2020; Khan et al. 2020).

The application of Cu NPs in plant species such as *Capsicum annuum* L., *Annona muricata* L., *T. aestivum* (Noman et al. 2020; Jonapá-Hernández et al. 2020; González-García et al. 2021), or CuO NPs in *L. sativa*, *Nicotiana tabacum* L., *Gymnema sylvestre* R.Br. (Mahjouri et al. 2018; Chung et al. 2019a; Xiong et al. 2021) increased content of phenols, flavonoids, or both.

In the case of iron-based nanoparticles such as Fe_2O_3 NPs, the phenol content in *Melissa officinalis* L. has been increased with the application to the soil (Mohasseli et al. 2020). While in *D. moldavica* the content of phenols and flavonoids increased with the application of NPs via foliar application (Moradbeygi et al. 2020). In *L. sativa* var. Foliosa Bremer, the foliar application of Fe_2O_3 NPs induced a greater accumulation of phenols, carotenoids and ascorbic acid (Jurkow et al. 2020).

Other NPs based on elements such as Al, Hg, Mn, Mo, Ni, Se, Si, Ti, and Zn have increased the content of phenolic compounds in various plant species such as *Nigella arvensis* L., *Z. mays*, *Atropa belladonna* L., *Cucumis sativus* L., *Brassica rapa* L., *C. annuum*, *Camellia sinensis (L.) Kuntze*, *Punica granatum* L., *Vicia faba* L., *T. aestivum*, *Oryza sativa* L., *H. reticulatus*, *D. kotschyi*, *Raphanus sativus* L., *Brassica oleracea* L., *Brassica napus* L., *Stevia reboudiana* Bertoni, and *Lupinus termis* L. (See Table 10.1). This confirms the impact of the application of NMs on the secondary metabolism of plants, in this particular case the phenylpropanoid pathway.

Carbon-based NMs also modify the synthesis of phenolic compounds, in *Ocimum basilicum* L. the foliar application of graphene oxide NPs induced a greater production of phenols such as estragol, methyl chavicol, germacreno D and linalool (Ganjavi et al. 2021). While the addition of MWCNT in MS culture medium induced an increase in the content of phenols, but also of alkaloids and carotenoids in *C. roseus* (Ghasempour et al. 2019). This same effect was observed in *Satureja khuzestanica* L. and *Thymus daenensis* Celak with the addition of MWCNT to the culture medium, since in both cases the content of phenols and flavonoids increased (Ghorbanpour and Hadian 2015; Samadi et al. 2020).

Chitosan nanoparticles have also been effective in increasing the content of phenolic compounds in various plant species. In *C. annuum* and *O. sativa* the foliar application of CS NPs induced a higher content of phenols (Taheri et al. 2020; Divya et al. 2020). In *C. sinensis*, the content of various phenols such as gallic acid, epicatechin, epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate were increased when CS NPs were applied through the nutrient solution (Chandra et al. 2015). While in *V. radiata* the application of CS NPs through seed priming increased the content of phenols and flavonoids, as well as carotenoids and ascorbic acid (Sen et al. 2020).

10.4.2 Impact of NMs on Ascorbic Acid

Ascorbic acid and glutathione are two of the main non-enzymatic antioxidant compounds in plants, and in addition to their ability to directly neutralize ROS, they are also cofactors for enzymes such as APX and GPX. These antioxidants are also increased by the application of various NMs.

The application of Ag NPs and Fe_2O_3 NPs through the foliar route induced a higher content of ascorbic acid in *L. sativa* (Jurkow et al. 2020; Wu et al. 2020). In *V. radiata* it was increased with the application of CS NPs through seed priming (Sen et al. 2020), in *C. annuum* with Cu NPs applied to the soil (Rawat et al. 2019). ZnO NPs also increased the content of ascorbic acid in *Glycine max* L. with foliar application (Ahmad et al. 2020), in *Solanum lycopersicum* L. applied to the soil (Akanbi-Gada et al. 2019), and in *L. termis* through seed priming (Abdel Latef et al. 2017).

The application of Fullerene through seed priming in *T. aestivum* also induced an increase in ascorbic acid (Shafiq et al. 2021). While in *Calendula officinalis* L. the same effect was observed when MWCNT was applied via foliar (Sharifi et al. 2021).

10.4.3 Impact of NMs on Carotenoids

It has been consistently reported that the application of NMs induces an increase in carotenoids. In other antioxidants, carotenoids are stimulated by the application of NMs through different pathways and with different types of NMs. The carotenoids content has increased through seed priming by the application of Al_2O_3 NPs in *H. sabdariffa* (Abdel Latef et al. 2020), SiO_2 NPs in *S. lycopersicun* and *Astragalus fridae* Rech.f. (Moghanloo et al. 2019; Madany et al. 2020), ZnO NPs in *T. aestivum* (Rai-Kalal and Jajoo 2021) and CS NPs in *V. radiata* (Sen et al. 2020).

In application directed to the root of plants, it has also been possible to increase the content of carotenoids with the application of $NiFe_2O_4$ NPs through the nutrient solution in *Hordeum vulgare* L. (Tombuloglu et al. 2019), Si NPs applied to the soil in *T. aestivum* (Ali et al. 2019b), ZnO NPs applied to the soil in *S. lycopersicum* (Ahmed et al. 2021). While via drench with ZnO NPs in *Arabidopsis thaliana* (L.), Heynh. (Wang et al. 2016) and with $ZnFe_2O_4$ NPs in *Pisum sativum* L. (Abdelhameed et al. 2021).

Through foliar application of NMs, it has been possible to consistently increase the carotenoids content. This has been observed in *D. moldavica* with CeO_2 NPs (Mohammadi et al. 2021), in *L. sativa* and *T. aestivum* with Fe_2O_3 NPs (Jurkow et al. 2020; Al-Amri et al. 2020), in *C. sinensis* and *Fragaria x ananassa* with Se NPs (Zahedi et al. 2019a; Li et al. 2021), in *Coriandrum sativum* L., *Linum usitatissimum* L. and *S. lycopersicum* with TiO_2 NPs (Raliya et al. 2015; Aghdam et al. 2016; Hu et al. 2020), and in *Phaseolus vulgaris* L. with ZnO NPs (Salehi et al. 2021).

It has even been reported that adding MWCNT to the MS culture medium increased the content of carotenoids as well as that of phenols and alkaloids in *C. roseus* (Ghasempour et al. 2019).

As has been shown in the literature, the application of NMs in plants definitely influences the synthesis and accumulation of non-enzymatic antioxidant compounds. With practically any type of NMs as well as by any application route, favorable results have been observed in plants (Table 10.1). This is due, as previously described, to the unique characteristics of NMs and their interaction with the various cellular structures that trigger a series of responses such as ROS production, modification in gene expression, and changes in metabolism, resulting in the production of a variety of compounds and metabolites, including non-enzymatic antioxidants.

10.4.4 Impact of NMs on Food Quality and Human Health

Antioxidants in general, especially non-enzymatic type, are very important for human health. This is because, as in plants, they have beneficial effects on human health such as reducing the risks of different chronic non-communicable diseases, and even reducing the risks of cancer (Saini et al. 2015; Cisternas-Jamet et al. 2020; Cortés-Estrada et al. 2020).

Since the fruits and vegetables that are consumed are the main source of bioactive compounds such as ascorbic acid, phenols, flavonoids, carotenoids, etc. (Arnao et al. 2001), then it becomes essential to improve the content of these compounds. NMs have the ability to induce the production of these compounds in plants (Table 10.1). Therefore, the application of NMs can be an excellent tool to improve the quality of plants that serve as food and thus induce positive effects on human health.

10.5 Conclusions and Prospects

Non-enzymatic antioxidants perform a series of fundamental functions for the development of plants, not only as antioxidants to neutralize ROS or RNS, but also other types of metabolic functions such as energy dissipation, or stress tolerance.

The production of these compounds can be modified by the application of various NMs and through different pathways, due to the ability of these materials to interact with cellular structures and overexpress different genes and metabolic pathways linked to the synthesis of non-enzymatic antioxidants. Thus, the use of NMs to regulate the synthesis of non-enzymatic antioxidants in plants can be a very important strategy to achieve different goals such as the production of foods with a higher content of bioactive compounds, or to improve the content of secondary metabolites that are of interest in industrial crops, or even crops with a greater capacity to tolerate biotic or abiotic stress.

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Chapter 11
2D-Nanosheets Based Hybrid Nanomaterials Interaction with Plants

Divya Chauhan, Mohammad Ashfaq, R. V. Mangalaraja, and Neetu Talreja

Abstract Agricultural growth needs a newer policy that speeds up plant growth and the nutritional value of the crops. Numerous agrochemicals, pesticides, and fertilizers provide nutrients to crops and enhance plant growth and nutrition quality. However, the demand for food remains a concern. In this context, 2D-nanomaterials or nanosheets have the potential ability to overcome issues associated with agrochemicals. 2D-nanosheets easily penetrate the seed coats and translocate with the plants using apoplastic and symplastic pathways. The high translocation ability regulates various molecular and biochemical pathways, thereby improving plant growth and development. However, a higher dose of the 2D-nanosheets shows the phytotoxic effects by increasing the production of reactive oxygen species. In this context, 2D-nanosheets-based hybrid materials might be beneficial for improved plant growth with minimal phytotoxicity. Moreover, 2D-nanosheets-based hybrid materials also protect crops against various pathogenic microorganisms. This book

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chapter focuses on synthesizing 2D-nanosheets, 2D-nanosheets-based hybrid materials, and their interaction with the plants. We also discuss the effect of 2D-nanosheets and 2D-nanosheet-based hybrid materials for plant growth and the protection of crops.

Keywords Crop protection · Nanofertilizers · Plant growth · Translocation · 2D-nanosheets

11.1 Introduction

Presently, the fast population growth and changes in the weather/climate globally require effective crop cultivation practices that fulfill increasing food needs. Efficient crop cultivation processes are one of the significant challenges now-a-days. Agricultural growth requires a new platform to accelerate plant growth and crop nutrition quality. Moreover, several materials in the form of pesticides and fertilizers are used to provide protection and nutrients to crops for enhanced plant growth and nutrition. These chemicals manage phytohormones levels within the plants that improve plant growth, crop yield, and the nutritional quality of grains. However, excessive use of agrochemicals decreases soil fertility and adversely affect the environment. Additionally, these chemicals accumulate in the plants and cause adverse effects to humans and animals health as well as negative impact on the environmental (Afreen et al. 2022b, 2022a; Ashfaq and Khan 2017; Chauhan et al. 2020; Sultana et al. 2022). To overcome these limitations, several nanomaterials have been synthesized and applied as nanofertilizers.

Nanomaterials (NMs) such as one-dimensional (1D) (carbon nanotubes (CNTs), carbon nanofibers (CNFs), metal-and metal-oxides), two-dimensional (2D) (graphene, graphene oxide (GO), and nanosheets of metal-and metal-oxide) nanomaterials have been used to augment plants growth. The 1D-nanomaterials like metal and metal-oxide (Cu, Zn, Fe, Ce, and Au) augmenting the growth of the plants. However, these metals and metal-oxides accumulate on the root surface causing less translocation ability and phytotoxicity (Ashfaq et al. 2022; Irsad et al. 2020; Kumar and Talreja 2019; Mustafa et al. 2011; Omar et al. 2019a, 2019b; Talreja and Kumar 2018). In this respect, carbon-based nanomaterials like CNTs, and CNFs are efficiently used in various applications, mainly antibiotics materials (Ashfaq et al. 2016), drug delivery (Ashfaq et al. 2014), environmental remediation [i.e., removal of heavy metals ions (Afreen et al. 2018; Khare et al. 2013; Kumar et al. 2011; Talreja et al. 2014), pharmaceuticals compounds (Saraswat et al. 2012; Talreja et al. 2016), and microorganism from water (Singh et al. 2013)], sensor (Ashfaq et al. 2018; Kumar and Talreja 2019), wound dressing (Ashfaq et al. 2017b; Bhadauriya et al. 2018), nanomedicine (Madani et al. 2011) and agriculture (Ashfaq et al. 2017a; Kumar et al. 2018). The CNTs and CNFs have the potential ability to penetrate seed coats, thereby efficiently translocate within the plants. The CNTs and CNFs also served as carriers for the agrochemicals or micronutrients, or fertilizers due to insignificant toxicity against both animal and plant cells (Ashfaq et al. 2013). Despite the tremendous success of the 1D materials, researchers continue focusing on the development of newer NMs that enhances the growth, development, and yield of the crops. In this aspect, 2D-nanomaterials have the potential ability to efficiently translocate within the plants and enhance the crops' growth and productivity. 2D-NMs are the newer class of material that has at least 1D in nanometer-sized. The 2D-NMs are widely used in several applications, including agricultural, energy storage, sensor, environmental decontamination, photocatalyst, biomedical, etc. due to their excellent property such as large surface area, feasible surface charge, high mobility in soil, biocompatibility makes it an ideal candidate (Ashfaq et al. 2021, 2022; Nag et al. 2018; Sasidharan et al. 2021, Sun et al. 2018, Tao et al. 2017; Wang et al. 2019; Ismail 2019; Zheng et al. 2021). One of the widely used members of this class is Graphene. Graphene was first exfoliated from graphite, a three-dimensional counterpart of graphene using the scotch tape method by Novoselov and Geim in 2004 (Cai et al. 2012); after that, several methods have been adopted to synthesize graphene. Few layered graphenes (FLG), ultrathin graphite, graphene oxide (GO), reduced graphene oxide (rGO), graphene oxide (GO), and graphene nanosheets (GNS) and their composites have been widely used in plant growth and development. Interestingly, these 2D-NMs like graphene and GO have translocation ability that easily penetrates seed coat due to the graphitic content and π-π electron. The higher translocation ability of the graphene and GO might be beneficial for the plants in various aspects, (1) increased the water uptake ability, (2) improved micronutrient delivery, (3) germination rate, (4) increased nutritional value of grains, and (5) plant growth and development. However, it seems difficult to used 2D-nanomaterials directly in the agricultural land due to handing that leads phytotoxicity. In this aspect, 2D-nanomaterials based hybrid materials might be resolved issues associated with 2D-nanomaterials.

Numerous polymers, such as polyvinyl alcohol (PVA), starch, cellulose, and chitosan, have been used to encapsulate 2D-NMs that efficiently delivered the agrochemicals or micronutrients or fertilizers (Sampathkumar et al. 2020; Sikder et al. 2021). These polymers aided various advantages like the controlled release of micronutrients or fertilizers or agrochemicals, increased soil nourishments, improved photosynthesis, water uptake capacity, and biocompatibility of the 2D-NMs. Herein, we discuss the synthesis of 2D-NMs, the interaction of 2D-NMs with plants, the role of 2D-NMs in the growth and development of plants, and the role of 2D-NMs based hybrid materials for plant growth. We also discuss the toxicity and prospects of 2D-nanosheets-based hybrid materials in agriculture.

11.2 Synthesis of 2D-Nanosheets

Recently, the synthesis of high-quality atomically thin 2D-NMs is attracting research interest as large areas and crystal quality enables the integration of optical and electronic devices with improved efficiency. Several challenges have to be overcome while synthesizing 2D material, such as thickness control, defects, size, crystal quality, and disorders; otherwise, detrimental consequences have been observed during device performances. Various synthetic approaches, including top-down and bottom-up, have been adopted to overcome these challenges, such as non-uniformity and defects in 2D crystals, which subsequently affect device performance (Han et al. 2015; Zavabeti et al. 2020). These methods have divided as follows.

11.2.1 Top Up Approach for the Synthesis of 2D-Nanosheets

The most widely used exfoliation techniques are part of the top-up approach. Exfoliations are of two types (1) liquid phase and (2) mechanical exfoliation. Liquid exfoliation is a straightforward, low cost and simple method. Nanosheets can be synthesized by placing the bulk materials into appropriate solvent and using sonication for some hrs. Solvent selection is one of the essential parts of liquid phase exfoliation is. The surface energy of solvent should be matched with the Vander wall interaction of nanosheet, which can improve the quality of exfoliation. Several kinds of literature use liquid-phase exfoliation to synthesize nanosheets, such as León-Alcaide et al. (2020) utilized the liquid-phase exfoliation method to separate Fe-based magnetic MOFs MUV-1-X, having a lateral dimension of 8 μm and thicknesses of 4 nm. The author observed nanosheets retain their structural integrity and magnetic properties. Huang et al. (2017) exfoliated SnSe nanosheet using liquid-phase exfoliation and the synthesized nanosheet is of high quality and can be utilized in photoelectronic applications (Huang et al. 2017). These studies suggest that liquid-phase exfoliation can be widely used. However, liquid-phase exfoliation also has several disadvantages, such as lower crystal quality and low yield limits its practical application (Huo et al. 2015). Figure 11.1 shows the schematic representation of the liquid exfoliation process.

11.2.2 Mechanical Exfoliation of 2D-Nanosheet

Novoselov et al. (2004) for the time used mechanical exfoliation to exfoliate graphene from graphite using scotch tape. After that, several 2D materials were synthesized using mechanical exfoliation. This method synthesizes high-quality 2D material with fewer defects and better crystal structure and controllability in patterned transfer. Huang et al. (2020) used Au-assisted mechanical exfoliation method and synthesized single-crystal nanosheet with high-quality crystal. He further made a theoretical study to confirm the method's applicability. Li et al. (2018) utilized mechanical exfoliation to exfoliate 2D perovskite microplates. The synthesized 20 nm thick nanosheet can be applied as electrodes for photodetection (Li et al. 2018).

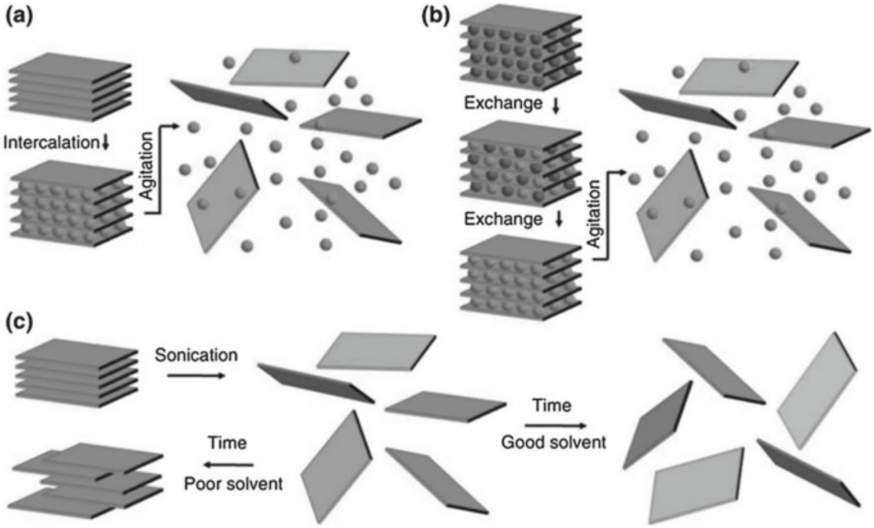

Fig. 11.1 Schematically illustration of the liquid exfoliation process, **a** by ions intercalation, **b** by ions exchange, and **c** by sonication. The figure was reproduced with permission (Huo et al. 2015)

11.2.3 Bottom-Up Method for Synthesis of 2D-Nanosheet

Chemical vapor deposition (CVD) is a potential method to grow nanosheets on various substrates such as nickel form, Cu foil, Activated carbon fabric, etc. The technique involves the decomposition of precursors on the substrate at high temperatures. This method synthesizes high-quality crystal, scalable size, tunable thickness, and excellent electronic properties with the effortless operation, which is considered an industrial-grade method. However, several parameters need to consider to achieve higher growth, such as temperature, pressure, flow rate, precursor gas, and substrate. Several researchers reported that there should be a balance between reactive gas and material (Okada et al. 2019; Seravalli and Bosi 2021; Zhang 2015). The CVD method was used to synthesize high-quality nanosheets, including the synthesize of $MoSe_2$ crystals on molten glass using CVD process within 5 min to achieve a triangular monolayer with lateral size of 2.5 mm and having carrier mobility up to ~95 $cm^2/(V \cdot s)$ (Chen et al. 2017). Another study synthesized a high-quality monolayer of WS_2 on a SiO_2/Si substrate using the CVD processsynthesized triangle domain of high crystal quality with 52 μm (Fu et al. 2015). Yan et al. synthesized HfS_2 flakes using the CVD process with a lateral size of 5 μm and thickness of 1.5 nm having hexagonal structure (Yan et al. 2017).

11.3 Interaction of 2D-Nanosheets with Plants

Usually, NMs or 2D-nanosheets easily penetrate the seed coats and translocate with the plants using two pathways, (1) Apoplastic pathway, which takes place outside of the plasma membrane (using cell-wall, extracellular spaces, and xylem vessels), and (2) Symplastic pathway, which take place within the cytoplasm (using plasmodesmata). Understanding the NMs or 2D-nanosheets translocation pathways within the plant is essential to indicate the accumulation or translocation path (Chichiriccò and Poma 2015; Sanzari et al. 2019a, 2019b; Su et al. 2019). The NMs or 2D-nanosheets are translocated within the plants through the xylem, indicating the translocation from root to shoot to leaf. In other words, NMs or 2D-nanosheets should be applied to the roots for better translocation. Whereas NMs or 2D-nanosheets show effective translocation through the phloem, indicating the foliar applications or downwards. In other words, NMs or 2D-nanosheets accumulate on the plant's fruits or grains. Moreover, translocation of the NMs or 2D-nanosheets is not limited to a specific cell (Cifuentes et al. 2010; Jeevanandam et al. 2018; Lin and Xing 2008; Spielman-Sun et al. 2019). The morphology and surface property of the NMs or 2D-nanosheets is mainly influenced the translocation ability. There are predominantly two characteristics is essential for the translocation of any NMs or 2D-nanosheets, (1) size of the NMs or 2D-nanosheets, up to 1 μm length of the NMs or 2D-nanosheets easily translocate within the plants. The nano-sized materials easily translocate within the plants through apoplastic and symplastic pathways. The NMs less than 50 nm in size easily translocate through the plasmodesmata. In contrast, larger size or more than 50 nm translocate through cell-wall, and (2) surface charge of the NMs or 2D-nanosheets, if the surface charge of the NMs or 2D-nanosheets is negative, so the negatively charged NMs or 2D-nanosheets interact with negatively charged plant cells, thereby NMs or 2D-nanosheets easily translocate within the plants through root to shoot to leaf due to strong repulsion force. On the other hand, if NMs or 2D-nanosheets are positively charged interact with negatively charged plants, thereby higher accumulation onto the root surface or less translocation ability due to solid attraction force (Ashfaq et al. 2017a; Kumar et al. 2018; Pérez-de-Luque 2017; Sultana et al. 2021). Figure 11.2 shows the translocation of the NMs or 2D-nanosheets within the plants through apoplastic and symplastic pathways. In general, smaller size and negatively charged NMs or 2D-nanosheets might be beneficial for practical translocation ability. Moreover, with the help of a functional group, we can easily modify the surface charge of the NMs or 2D-nanosheets. The higher translocation ability of the or 2D-nanosheets efficiently enhanced the plant growth, development, and yield of the crops by increasing protein content, chlorophyll content, root-shoot length, germination rate, and water uptake ability.

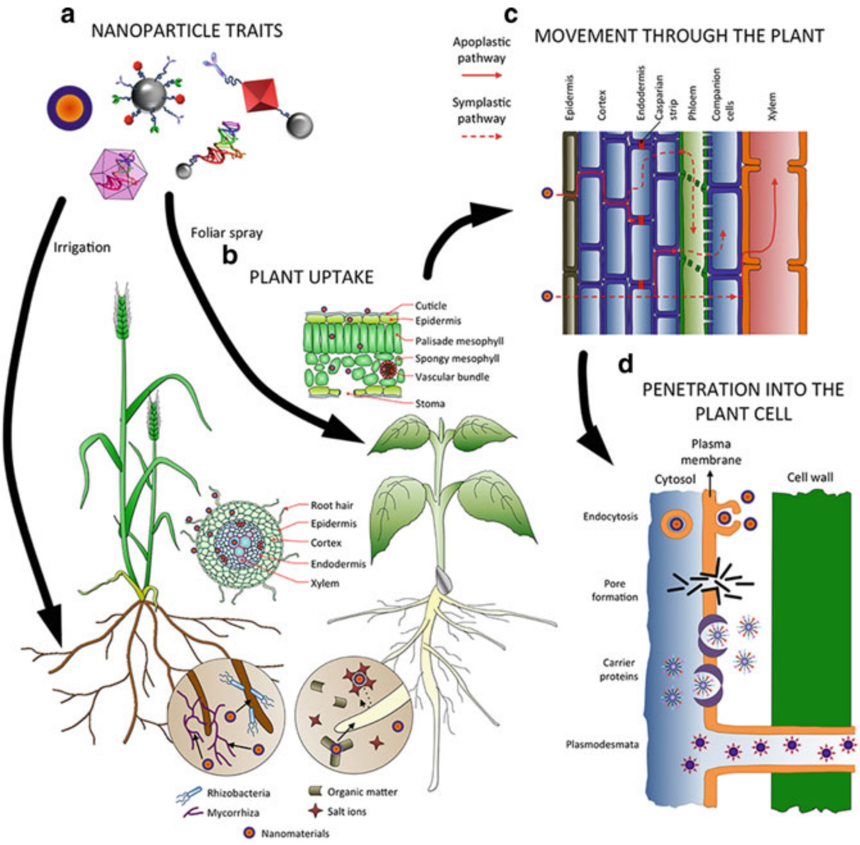

Fig. 11.2 Translocation of the NMs or 2D-nanosheets within the plants through apoplastic and symplastic pathways. The figure was reproduced with permission (Pérez-de-Luque 2017)

11.4 2D-Nanosheets for Plant Growth

The 2D-nanosheets research and development facilitate the next-generation delivery of micronutrients, agrochemicals, fertilizers, or pesticides within the plants. With the help of NMs or 2D-nanosheets, the plant's growth easily improved by enhancing water uptake capacity, chlorophyll content, protein content, and translocation ability of the micro-nutrients. These 2D-nanosheets extensively used in drug delivery, sensor, nanomedicine, environmental remediation, energy, and agricultural applications due to their exceptional characteristics (Aïssa et al. 2015; Carvalho et al. 2021; Choi et al. 2010; Dhinakaran et al. 2020; Hu and Zhou 2014; Mbayachi et al. 2021). Numerous 2D-nanosheets, especially graphene and graphene oxide (GO), have been used to augment plant growth. For example, Zhang et al. (2015) tested graphene against tomato seed and suggested that it has the potential ability to penetrate the seed coat; thereby, translocation was effectively observed. Moreover, the higher translocation ability of the graphene increased germination rate and seedling growth. The study suggested that graphene's remarkable translocation ability makes it a potential candidate for agricultural application (Zhang et al. 2015). Zhang et al. used graphene and tested it against wheat plants to determine graphene's toxic effects at higher concentrations. The data suggested that graphene shows the toxic effect at higher doses that induced oxidative stress, decreased chlorophyll content, and photosynthesis (Zhang et al. 2016). He et al. used GO and tested against spinach seeds. The data suggested that the GO increased the germination rate and growth of the plants. The lower dose of GO increased the plant growth, whereas higher doses showed adverse effects. Interestingly, GO increase the water uptake ability, oxygen content and efficiently translocate within the plants. Additionally, GO does not show any phytotoxic effect at a lower dose that makes promising materials for agriculture (He et al. 2018). Xie et al. use GO, and IAA-GO and tested against *Brassica napus* L. The data suggested that the GO inhibits the development of the roots. The co-treatment of phytohormones IAA-GO increased the inhibition rate. The exposure of GO increased the abundance of 1-aminocyclopropane-1-carboxylic acid synthase 2 (ACS2). The data suggested that the GO easily modulates various pathways to control the plants' growth (Xie et al. 2020). Park et al. synthesized GO using a chemical oxidation process and tested it against *Arabidopsis thaliana* L plants. The data suggested that the GO shows the constructive effects on the plants in terms of enhancing the length of roots, rate of flower bud formation, number of leaves, and area of leaves. Moreover, GO enhanced the size of fruits and ripening process, ultimately sweeter fruits than control plants fruits (Park et al. 2020). Mahmoud and Abdelhameed synthesized GO, lysine-GO (L-GO), and methionine-GO (M-GO) and tested against pearl millet (*Pennisetum glaucum* L.). The data suggested that the GO, L-GO, M-GO shows significant effect on the plants in terms of increasing the biomass accumulation, growth of the plants, photosynthetic pigments, and yield of the crops. Moreover, reducing reactive oxygen species (ROS) in plants under stress conditions (Mahmoud and Abdelhameed 2021). Figure 11.3 shows the photographic images of the pearl millet with exposure of GO, L-GO, and M-GO. Another study suggested focusing on the synthesis of GO and tested against different plants, mainly radish, alfalfa, lettuce, perennial ryegrass, and cucumber seeds. The data suggested that GO shows positive effects against radish, alfalfa, perennial ryegrass, and cucumber seeds. However, GO decrease the germination rate at lettuce plants. Moreover, phytotoxic effects were observed at a higher concentration of GO exposure. The data suggested that phytotoxicity depends on various factors like the amount of nanomaterials, plants species, and types of nanomaterials (Lee et al. 2021). Table 11.1 shows the comparative data of 2D-nanosheets, mainly graphene and GO, and its effects on different plants. The data suggested that the graphene and GO effectively increased the growth of the plants by increasing various factors like increased the root length, shoot length, biomass, chlorophyll content, protein content, photosynthesis process, water uptake ability, and germination rate. In general, 2D-nanosheets mainly graphene and GO has a potential ability that translocates within the plants. The higher translocation ability of the 2D-nanosheets improved growth and yield of the crops. Moreover,

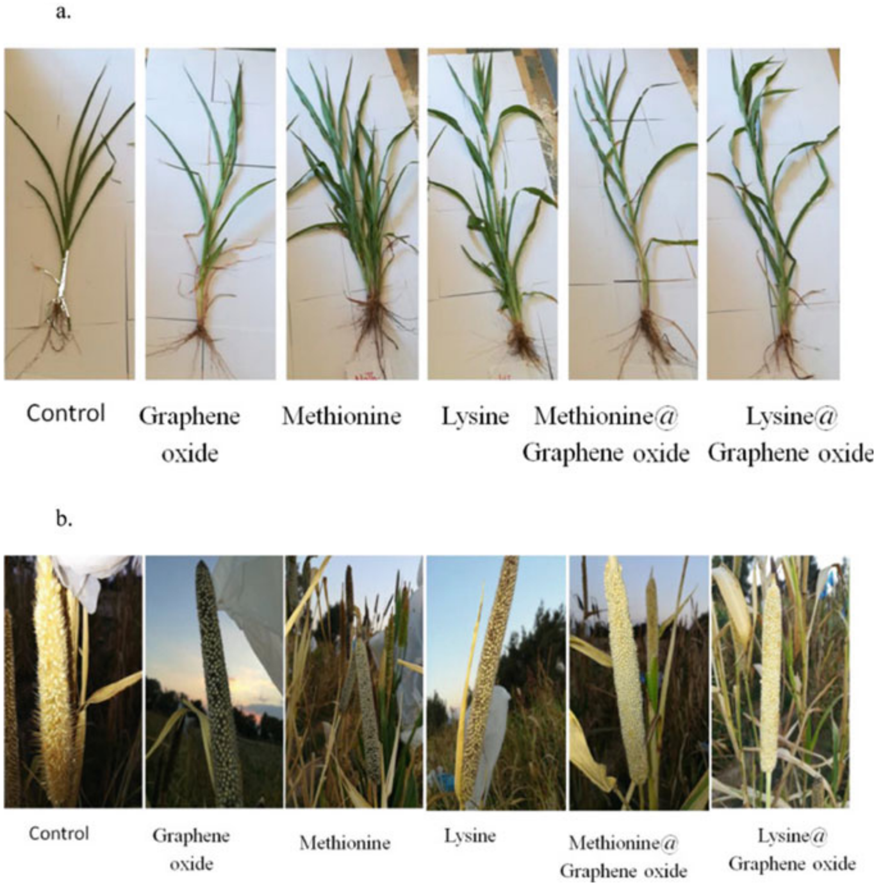

Fig. 11.3 Photographic images of the pearl millet with exposure of GO, L-GO, and M-GO. The figure was reproduced with permission (Mahmoud and Abdelhameed 2021)

the 2D-nanosheets do not show any toxicity at lower doses. However, high concentrations might show adverse effects in some plant species. The phytoxicity of the 2D-nanosheets mainly depends on various factors like types of nanomaterials, and types of plant species. Moreover, negatively charged graphene and GO effectively translocate within the plants.

Table 11.1 Different 2D-nanosheets and their effects on the plant growth

Nanosheets	Synthesis process	Plants	Effect of interaction	References
Graphene	Commercial	Tomato	Increased the germination rate	Zhang et al. (2015)
Graphene	–	Wheat	Induced oxidative stress, decreased chlorophyll content	Zhang et al. (2016)
GO	Commercial	Spinach	Increased the germination rate	He et al. (2018)
GO	Commercial	*Brassica napus* L	Inhibit the development of root	Xie et al. (2020)
GO	Chemical oxidation	*Arabidopsis thaliana* L	Increased the root length, fruit size, and sugar content	Park et al. (2020)
GO	Chemical oxidation	Pearl millet	It is increased plant growth, photosynthetic pigments, and biomass accumulation	Mahmoud and Abdelhameed (2021)
L-GO	Chemical oxidation	Pearl millet	It is increased plant growth, photosynthetic pigments, and biomass accumulation	Mahmoud and Abdelhameed (2021)
M-GO	Chemical oxidation	Pearl millet	It is increased plant growth, photosynthetic pigments, and biomass accumulation	Mahmoud and Abdelhameed (2021)
GO	Chemical oxidation	Lettuce, radish, alfalfa, perennial ryegrass, and cucumber seeds	Positive effects on plant growth. GO shows the negative impacts on lettuce	Lee et al. (2021)

11.5 2D-Nanosheets Based Hybrid Materials for Plant Growth and Protection

The 2D-nanosheets, mainly graphene and GO, are extensively used in agricultural applications. However, 2D-nanosheets shows toxic effect at higher doses that remains a concern. The researcher still focuses on modifying 2D-nanosheets for various aspects like incorporating the metal-nanoparticles and polymers that might improve the applicability and reduce the toxicity concern. Numerous 2D-nanosheets-based hybrid materials have been used for the improving growth and development of the plants (El Miri et al. 2016; Facure et al. 2017; Salim et al. 2021; Soraki et al. 2021; Rashidi Nodeh et al. 2017). For example, Zhang et al. synthesized fertilizers incorporated KNO_3 encapsulated graphene (F-K-Graphene) based hybrid materials and tested them for crop production. The data suggested that the F-K-Graphene-based hybrid materials release fertilizers in a controlled manner that might dramatically enhance the productivity of the crops (Zhang et al. 2014). Ren et al. synthesized sulfonated graphene and tested it against maize seedlings to determine oxidative stress at different concentrations. The data suggested that the sulfonated graphene increased the plant's growth at lower doses, whereas at higher doses decreased the development of the plants. Moreover, the rise in sulfonated graphene produces reactive oxygen species, reduces the soluble proteins, increases the enzymatic activity and intracellular Ca ions (Ren et al. 2016). Huang et al. synthesized C-14 labeled graphene (C-14-Graphene) and tested against rice plant. The data suggested that the C-14-Graphene increased the transformation and uptake within the plants that grew the plant growth and development (Huang et al. 2018). Soraki et al. synthesized Ag-nanoparticles and graphene-based hybrid materials and tested them against *Melissa officinalis.* The data suggested that the expression of synthase gene upon exposure to Ag-nanoparticles and Ag-graphene-based hybrid materials. Moreover, lower doses effectively induce the various molecular and biochemical pathways that enhance the growth of the plants (Soraki et al. 2021). Another study of the different group synthesized the similar Ag-graphene hybrid material and tested against *Stevia rebaudiana.* The data suggested that the Ag-graphene-based hybrid materials effectively increased the chlorophyll content, protein content, falvonoide content, accumulation of soluble sugar content, and total phenols. Moreover, the regulation of various molecular and biochemical pathways enhanced the growth and development of plants (Nokandeh et al. 2021). The above literature study suggested that the 2D-nanosheets-based hybrid materials effectively increased the growth of the plants and the yield of the crops. However, higher doses of the hybrid materials show the adverse effect that increased the production of reactive oxygen species. On the other hand, 2D-nanosheets-based hybrid materials also protect the crops against various pathogens. Several studies suggested that 2D-nanosheets-based hybrid materials effectively kill or protect the crops against pathogens. For example, Chan et al. synthesized Ag-graphene-based hybrid materials and tested them against *Fusarium graminearum.* The data suggested that the synthesized Ag-graphene-based hybrid materials efficiently inhibit the fungus in both *in-vitro* and *in-vivo.* The spore germination was inhibited even at a lower concentration of the hybrid materials. The mode of action of the hybrid materials might be increasing the production of reactive oxygen species and physical injury (Chen et al. 2016). Figure 11.4 shows the synthesis of Ag-graphene-based hybrid materials and their antifungal activity. Li et al. synthesized borneol-GO (B-GO) based hybrid materials and tested them against M. racemosus. The data suggested that the B-GO effectively inhibits the spore germination of the fungus. Moreover, no growth was observed up to five days of exposure, indicating the exceptional antifungal agents (Li et al. 2017). El-Abeid et al. synthesized Cu decorated reduced-GO (Cu-rGO) based hybrid materials and tested them against fusarium and wilt diseases of tomato and pepper plants. The data suggested that the Cu-rGO based hybrid materials effectively inhibit fungus growth and reduce wilt diseases without any phytotoxic effects up to 70 days (El-Abeid et al. 2020). Table 11.2 summarizes the comparative data of the different 2D-nanosheets-based hybrid materials and their impact on plant growth and crop protection. The study suggested that 2D-nanosheets-based hybrid materials effectively increased the growth and development of the plants by improving various molecular and biochemical pathways. Moreover, these 2D-nanosheets-based hybrid materials do not show any phytotoxic effects at lower doses, whereas some of the 2D-nanosheets-based hybrid materials show toxic effects at higher doses. The phytotoxicity of the 2D-nanosheets-based hybrid materials depends on various factors like types of 2D-nanosheets-based hybrid materials, plants, and species of the plants. 2D-nanosheets-based hybrid materials show lesser toxic effects compare with that of the 2D-nanosheets. Interestingly, the high translocation ability of the 2D-nanosheets-based hybrid materials aided advantageous that improved the delivery of micronutrients or fertilizers or agrochemicals, thereby high yield of the crops. Besides the growth and development of plants, 2D-nanosheets based hybrid materials are also effectively used for the protection of crops against various pathogens, as numerous polymers and metals have exceptional antimicrobial activity. In general, with the help of the 2D-nanosheets based hybrid materials we can easily improve plant growth as well as crop protection.

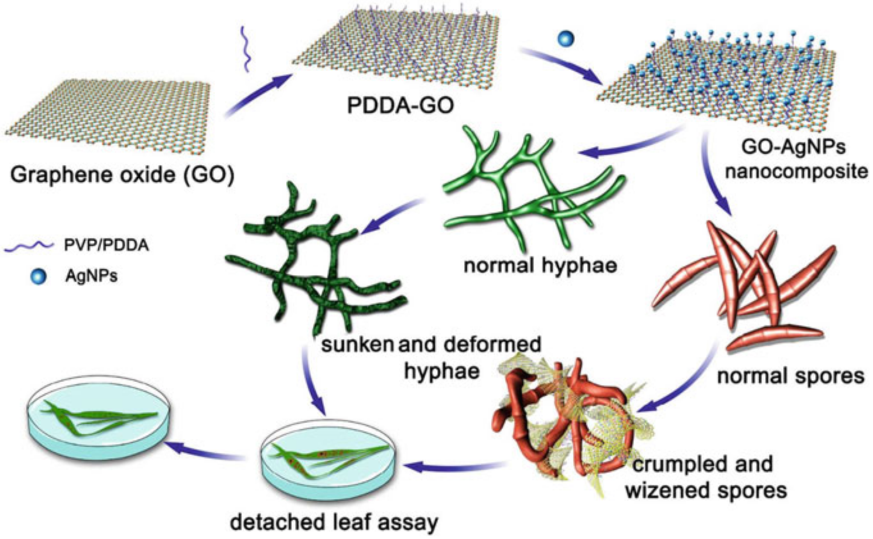

Fig. 11.4 Schematic representation of the synthesis of Ag-graphene-based hybrid materials and their antifungal activity. The image was reproduced with permission (Chen et al. 2016)

Table 11.2 Different 2D-nanosheets-based hybrid materials for plant growth and crop protection

Hybrid materials	Plants/Pathogens	Effects	References
Ag-graphene	*Melissa officinalis*	Low doses induce molecular and biochemical pathways	Soraki et al. (2021)
Ag-graphene	*Stevia rebaudiana*	Increased chlorophyll and protein content	Nokandeh et al. (2021)
Ag-graphene	*Fusarium graminearum*	Inhibit phytopathogens	Chen et al. (2016)
B-GO	*M. racemosus*	Inhibit fungal growth	Li et al. (2017)
Cu-rGO	Fusarium and wilt diseases	Inhibit spore germination	El-Abeid et al. (2020)
C-14-Graphene	*Rice*	Increased uptake and transformation	Huang et al. (2018)
F-K-GO	–	Controlled release of fertilizers	Zhang et al. (2014)
Sulfonated-graphene	*Maize*	Lower doses increased the plant growth, whereas higher concentration decreased	Ren et al. (2016)

11.6 Conclusion and Prospects

Tis chapter has addressed the synthesis of 2D-nanosheets or NMs, the interaction of 2D-nanosheets with plants, the role of 2D-nanosheets in the growth and development of plants. The effect of 2D-nanosheets-based hybrid materials for plant growth and crops protection was also discussed. The nano-sized and negatively charged 2D-nanosheets might be beneficial for effective translocation ability. Additionally, with the help of a functional group, tuning the surface charge of the 2D-nanosheets can be easily achieved. The higher translocation ability of the NMs or 2D-nanosheets efficiently enhanced the plant growth, development, and yield of the crops by increasing protein content, chlorophyll content, root-shoot length, germination rate and water uptake ability. 2D-nanosheets-based hybrid materials are also effectively used for the protection of crops against various pathogens. Therefore, the 2D-nanosheets-based hybrid materials can potentially improve plant growth and crop protection that might be next-generation tools for agriculture. Polymeric delivery system might be beneficial for the real-time application of NMs.

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Part III
Agricultural Implications

Chapter 12
Nanomaterial Impact on Plant Morphology, Physiology and Productivity

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Abstract Nanoparticles (NPs) have a remarkable impact on plants. Plants respond to NPs in many ways, including stimulation and inhibition. The responses could be clearly observed via the changes in plants' morphological, physiological, and productive indicators. This chapter focuses on the morphological changes in plants and seedlings' growth and the fresh and dry weight of plants and seedlings. In addition, the chapter concentrates on the number and lengths of roots, shoots, and leaves. Different modifications that occur due to NPs' influence on flowers, pods, and grain are also covered. The chapter further discusses the interaction mechanism of NPs with seed germination, plant development, and reproduction by interacting with plant cells' surfaces. The biochemical interaction series that could stimulate the plants internally are also discussed. Furthermore, the chapter provides details on the negative and positive effects of NPs on various plant parts, including root, stem, leaf, flower, and fruit. The impacts of different nanomaterials (NMs) include carbon, titanium dioxide, silver, zinc oxide, copper oxide, silica, cerium dioxide, aluminum oxide, selenium, gold, fullerene, and iron, on plants are demonstrated in this chapter. The material's particle size, concentration as well as plant species are also taken into account. All the previously mentioned effects demand more research to realize the mechanisms that occur in plants as a result of treatment with various NPs.

Keywords Fruits · Leaves · Nanoparticles · Nanoparticle response · Plant morphology · Root growth · Seed germination · Stems

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12.1 Introduction

Humans have intelligently utilized plant organs for a variety of uses, including food and medicine (fruits, seeds, roots, stems, and leaves), clothes (flowers), furniture (trunks), and paper (trunks). In addition, plant leaves absorb carbon dioxide and produce renewable oxygen (Ali et al. 2021). These plant organs are affected by numerous factors such as temperature, light, nutrients, and water (Patil et al. 2021, Shafiq et al. 2021).

The study of the shape, size and placement of plant organs such as seeds, roots, stems, leaves, flowers, and fruits is known as plant morphology (Hossain et al. 2020). Recent researchers have demonstrated the significant impact of nanotechnology on plant morphology, including particle size, shape, and material concentration (Singh et al. 2015). These variables might influence not only plant morphology but also seedling germination and phytotoxicity. As a result, nanotechnology can alter the present synthetic framework used in modern agriculture systems (Arora 2018, Kerry et al. 2017; Prasad et al. 2017; Shang et al. 2019; Usman et al 2020).

This chapter describes the influence of NPs on plant morphology. It covers the mechanism of seed interaction with NPs, explains the recent experimental results of the effect of NPs on seed germination and root growth, and discusses how NPs of different elements and their oxides affect the morphology of stem, leaves, flowers, and fruit.

12.2 Mechanism of NPs Interaction in Seed

The plant growth inhibitory or stimulatory effects are produced by interacting between the surface charges of NMs/NPs and the surface charges of plant cells. However, that interaction with metal NPs differs depending on the features of NPs, such as the metal nature, concentrations of NPs, phase growth, and plant species (Pérez-de-Luque 2017).

NPs can induce seed germination and develop many parts of a plant (Juárez-Maldonado et al. 2019). Seed germination is the foundation-initiated stage for plants' growth, development, and productivity (Hossain et al. 2020). NPs-treated seeds achieve high germination by improving seed absorption and water retention (Juárez-Maldonado et al. 2019) (Fig. 12.1).

The bio-stimulation effect is demonstrated in two stages. The first stage is implemented via the interaction of surface charges of a physicochemical nature. Following this stage and as a second stage, a series of biochemical stimuli are triggered via the entry of NPs and NMs into the plant cells due to alteration of the cellular membrane (Ali et al. 2021). Figure 12.2 shows the most probable effect of nanoparticles on plant parts.

12.3 Effect of NPs on Seed Germination and Root Growth

12.3.1 Carbon-Based NPs

Utilizing carbon-based nanomaterials (CNTs) raised water uptake for the seed via passing it from the seed coat and then reaching to shoots and leaves (Omar et al. 2019) which favorably affects the germination percentage and plant growth via capitalizing on the efficiency of water uptake moreover increasing some substantial nutrients uptake (Singh et al. 2015). This stimulatory effect can be explained by producing microspores by the CNTs (Ali et al. 2021; Sanborn et al. 2018). This mechanism has been applied to enhance seed germination and root growth, and final plant growth for several crops, such as hybrid Bt cotton, *Phaseolus mungo* L., *Brassica juncea* L., tomato (*Lycopersicum esculentum* Mill), and rice (*Oryza sativa* L.) which increased the biomass of rice plants and the germination rate of seeds by 90% within 20 days, compared with 71% in the control sample (Ali et al. 2021).

The results of the research show the role of CNTs as a promoter of rice seedling growth, activation effects on root elongation, and seed germination in zucchini species (Aslani et al. 2014). In addition10-40 mg/L CNTs significantly increased seed germination, vegetative biomass, and tomato plant growth (Remedios et al. 2012). Also, the influence of MWCNT (50 μg/ml) on tomato roots was observed in fresh and dry mass and gene expression variety (Predoi et al. 2020).

In addition, MWCNTs increased the germination of previously treated seedlings, as confirmed by transmission electron microscopy (TEM) and Raman spectroscopy (Singh et al. 2015).

As well as the treatments by single-walled carbon nanohorns (SWCNHs) increased seed germination in some crop species: maize, tobacco, switchgrass, rice, tomato cell cultures, Barley, Wheat, and soybean (Ali et al. 2021). On the contrary, no effect was found for treatment by SWCNTs (84 h) for roots of cucumber seedlings (Aslani et al. 2014).

12.3.2 Titanium Dioxide NPs

The impact on the morphology of plants is varied on the type of NPs and the method of application (Hossain et al. 2020). However, the essential key to promoting the seed germination rate is the permeation of NMs into the seed (Aslani et al. 2014). The best effects were at 2500 mg/L when applying the concentrations from 2500 to 40,000 mg/L to senescent seeds.

Titanium dioxide NPs have promoted seed germination through their more capacity to the carriage of water to the internal tissues and increased the metabolism of the seed reserves as indicated for the NPs of Ag and graphene (Juárez-Maldonado et al. 2019).

At 250–4000 mg/L, TiO_2 nanoparticles substantially increase the germination rate and germination index of naturally aged spinach seeds. TiO_2 NPs increases the seedling's dry weight and seedling vigor index considerably. (Predoi et al. 2020). In addition, titanium dioxide NPs facilitate water absorption and consequently quicken seed germination (Ali et al. 2021).

Treated seeds with TiO_2 NPs provided plants with three times higher photosynthetic rates, 73% more dry weight, a 45% increase in chlorophyll compared to the control over the germination period of 30 days (Aslani et al. 2014).

When spinach roots are exposed to TiO_2 NPs, increased plant growth has been observed by improving nitrogen metabolism that promotes the adsorption of nitrate and photosynthetic rate (Predoi et al. 2020). There was a correlation between the growth rate of spinach and the size of the materials, so the smaller NMs produce better germination (Siddiqui et al. 2020). Foliar application with 20 g/L TiO_2 NPs increased root and stem length, ear mass biomass, flowering, and seed number of wheat (Predoi et al. 2020).

Application of TiO_2 NPs for seedlings plants canola stimulated the growth of radicle and plumule, root, and seed germination. However, it inhibited root elongation in cucumber (Khana et al. 2019). Titanium dioxide NPs at 60 mg/L (bulk and nanosized) encouraged seed germination percentages of the sage plants. This concentration could gain the lowest mean germination time. However, higher concentrations did not do that. Therefore, the vigor index of sage was raised by using TiO_2 NPs to seeds compared to the control and bulk TiO_2 treatments (Aslani et al. 2014).

12.3.3 Silver NPs

Silver NPs had positive or negative effects on vascular plants, such as seed germination, root growth, and plant biomass. These effects were related to the concentration and the shape of NPs (Aslani et al. 2014). Furthermore, these effects may be attributed to chemical precursors. Therefore, plant extractions are involved in the biosynthesis method which is widely used to synthesize NPs. Figure 12.1 illustrates the schematic presentation for biosynthesized Ag NPs using leave extraction. The approach demonstrated well-controlled particle concentration and size (21–42 nm), as well as particle dispersion. They are spherical in shape and uniformly distributed (Bamsaoud et al. 2021). This method may aid in reducing the negative effects of NPs.

Many researchers have observed the effects of silver NPs on plants growth. For example, silver NPs show harmful effects on seed germinations, root, and shoot growth on species of Chinese cabbage (*Brassica campestris*), rice (*Oryza sativa* L.), and Mung bean (*Vigna radiata* L. Wilczek) at concentrations of 3000 g/mL, 4500 g/mL and 6000 g/mL, respectively (Yan and Chen 2019). On the other hand, silver NPs positively affected seed germination and the root growth of zucchini plants in hydroponic solution. At the same time, it observed a decrease in plant biomass and transpiration in the presence of Ag NPs (Aslani et al. 2014).

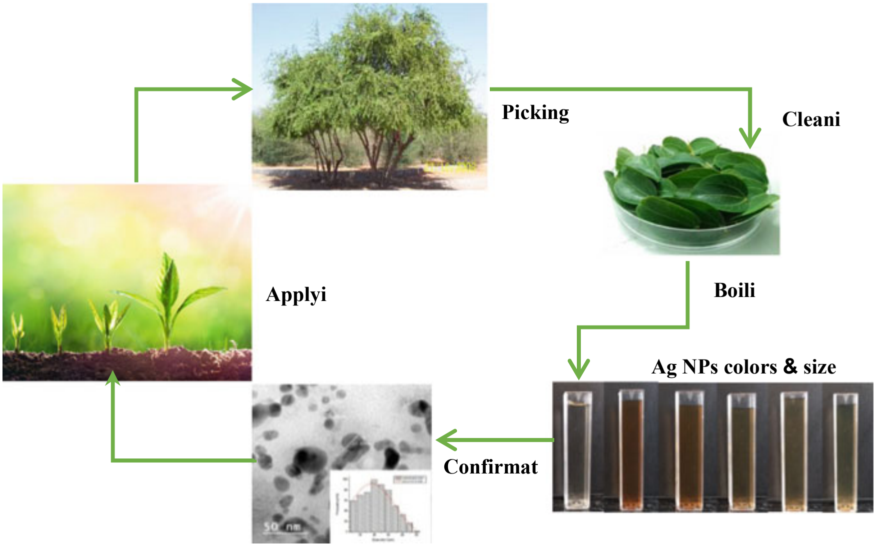

Fig. 12.1 Schematic presentation for biosynthesis, characterization, and plant application of synthesized silver nanoparticles using leaf extract

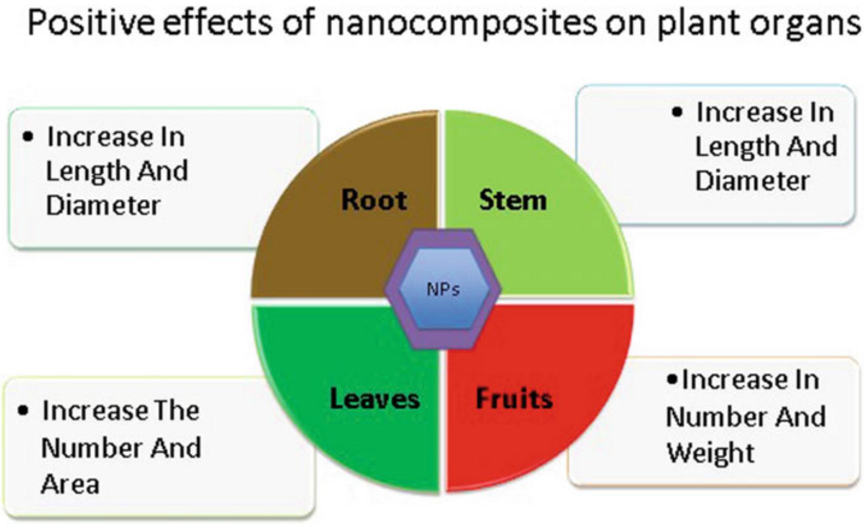

Fig. 12.2 The most probable effect of nanoparticles on plant parts

It has been certified that Ag NPs with sizes of up to 29 nm had harmful effects on the germination of cucumber seeds and lettuce. Still, no toxic effect has been observed on the germination of barley and ryegrass exposed to Ag NPS (Yan and Chen 2019). The number of studies about the effect of Ag NPS in two varieties of wheat and barley noted an increase in germination ratio stem length and reduced length root compared to the control (Al-Hadede et al. 2020).

Treatment with 75 ppm Ag NPs application on wheat plants resulted in a negative response for fresh root weight and root length. At the same time, a positive response was observed to cowpea (50 ppm) and brassica plants (Yan and Chen 2019). Silver NPs promoted growth and increased root nodulation. Low concentrations of Ag NPs (10–20 g/mL) improved seedling development and seed germination in fenugreek plants (Predoi et al. 2020).

12.3.4 Zinc Oxide NPs

Plants required low concentrations of ZnO NPs for the normal developmental process. On the other hand, higher levels of Zn in plants can cause toxic effects such as inhibition of cell elongation and division, the reduction of growth and plant biomass, curling and rolling of young leaves, chlorotic and necrotic leaf tips, wilting, and root growth inhibition (Predoi et al. 2020). For example application of ZnO NPs resulted in a dose-dependent inhibition of seed germination in cabbage. During seed germination in wheat, lower concentrations of ZnO NPs were more beneficial. However, the lower concentration does not inhibit seedling growth and cell division in onions (Khana et al. 2019).

Similarly, the germination of cucumber seeds increased 10% by ZnO-NPs compared to the control (Velasco et al. 2020). In addition, lower concentrations of ZnO NPs improve seed germination in soybean, wheat, tomato, and onion. Also, ZnO NPs with (50 nm) particle size positively affected the rooting of rapeseed in contrast with the impact of Zn^{+2} ions (Khana et al. 2019).

Using ZnO NMs on different plants increased root length (4.2%), shoot length (15.1%). In addition, ZnO NPs application on the coffee plants had a positive effect by increasing the fresh weight of roots (37%) (Predoi et al. 2020).

The biogenic ZnO-NPs influenced the shoot and root length of maize seedlings at 14 DAS. The treatment which effectively increased root length was 25 mg/L (T25). In contrast, the 200 mg/L (T200) concentration of ZnO-NPs exerted an inhibiting effect (Buono et al. 2021).

Biological synthesis of ZnO NPs has been prepared using brown seaweed *Turbinaria* ornata. (Turner) J. Agardh extracts to promote rice seed quality and crop yield. ZnO NPs at (10 mg/L) showed that they have been prompt in the seed germination (100%), root length (185 mm) root width (0.5 mm) compared to control (Itroutwar et al. 2019).

12.3.5 Copper Oxide NPs

Cu NPs up to 1000 mg/L have detrimental impacts on the seedling growth of mung bean (Vigna radiata L. Wilczek) and wheat, and may reduce the biomass of zucchini by 90% relative to the control compared to the higher concentration (Omar et al. 2019). Also using higher concentrations of Cu NPs reduce shoot and root growth of soybean, decrease germination rate and biomass in Oryza sativa L. and it could inhibit seed germination in cucumber (Predoi et al. 2020). On the other hand, it was indicated that soil amendments with metallic Cu NPs up to 600 mg/kg significantly increased lettuce seedling growth up to 91% without toxic effects (Omar et al. 2019).

The *Sesbania virgata* (Cav.) Pers seeds were subjected to different concentrations of CuO NPs. The results showed that CuO NPs induced a considerable change in seed temperature and a reduction in root length. This was signalized metabolic damage and changes in energy dissipation and plant growth (Santos et al. 2021).

12.3.6 Iron Oxide NPs

The research results showed that nα-Fe_2O_3 was effective on seed germination (89.17%) due to the role of iron in germination and increasing biomass of *Oenothera biennis L.* (Asadi-Kavan et al. 2020). Furthermore, nano zero-valent iron utilized promoted the elongation of the root system in *A. thaliana* (Khana et al. 2019). Predoi et al. (2020) indicated that the foliar and root usage of Fe_2O_3 NPs increase root elongation.

12.3.7 Silica NPs

Lower amounts of nano SiO_2 (in the concentration of 8 g/L) increased the germination of seeds in tomatoes by 22.16%, mean germination time, seed germination index, seed vigor index, fresh seedling weight, and dry weight. In addition, with a significant impact on root growth through the main length of roots, seedlings lateral root number, and diameter of root collar (Predoi et al. 2020). Similarly, rice seed germination was induced with Si NPs, while quantum dots arrested the germination (Aslani et al. 2014).

Increases in seed germination caused by Si NPs in maize are related to enhanced nutritional availability to seeds (Singh et al. 2015). For example, in *Changbai larch* (*Larix olgensis* Henry) seedlings, Si NPs improved seed germination traits, including percent germination and germination rate, length, fresh and dry mass of root and shoot (Siddiqui et al. 2020). Also, in tall wheatgrass (*Thinopyrum intermedium* L.), using Si NPs for Pre-chilling seeds breaks inertia, promotes seed germination, and increases vigor index, mean germination time, and dry weight seedling roots and shoots (Al-Hadede et al. 2020).

Additionally, using Si-NPs in seed priming and seed soaking increased seedling biomass and vigor index along with seedling root and shoot length of *Helianthus annuus* L. (Omar et al. 2019). Germination and growth of soybean (*Glycine max* L.) were improved by increasing nitrate reductase activity and enhancing seeds' ability to absorb and utilize water and nutrients (Siddiqui et al. 2020). The positive effects on seed germination, length, and dry weight of root and shoot in rice (Oryza sativa L.) seedlings were observed when Si-NPs were used (Elshayb et al. 2021).

12.3.8 Cerium Dioxide NPs

The results of the effect of CeO_2 on seeds of tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), and corn (*Zea mays* L.) found that CeO_2 NPs (2000 mg/L) meaningfully decreased corn germination (about 30%). The germination of tomato and cucumber was reduced by 30 and 20%, respectively (Ali et al. 2021). On the other hand, adding cerium dioxide, NPs can raise plant biomass and prompt anthocyanin production, yet showed little impact on root lengthening (Khana et al. 2019).

12.3.9 Aluminum Oxide NPs

Al_2O_3 NPs at concentrations up to 4000 mg/L had no significant toxic effects on seed germination, root elongation of Arabidopsis thaliana L. (Remedios et al. 2012). Al_2O_3 NPs (The aqueous suspension) improved the root growth of radish. On the contrary, the root growth decreased in cucumber (Hossain et al. 2020).

12.3.10 Selenium NPs

Correlation of Se NPs with selenate in *Nicotiana tabacum* L. showed that Se NPs invigorated organogenesis and expanded the advancement of the root by up to 40% compared to the impact of aqueous selenate (Khana et al. 2019).

12.3.11 Gold NPs

Maize aged seeds' exposure to photosynthesized gold NPs (5–15 mg/L) significantly improved their germination and physiology without any toxicity (Elemike et al.

2019). In addition, enhanced seed germination in (*Boswellia ovalifoliolata* Balakr and A.N. Henry) (Singh et al. 2015), *Arabidopsis thaliana* L. (Remedios et al. 2012), lettuce and cucumber, *Brassica juncea* L. and *Gloriosa superba* L. (Predoi et al. 2020). On the other hand, it resulted in cultures of barley with addition Au NPs decreased biomass, yellow leaves, and dark roots (Khana et al. 2019).

12.4 Effect of NPs on Shoots

In botany, the stem is the plant axis that carries buds and shoots with leaves and roots at its base. The stem's primary duties are to sustain the leaves by transporting the leaves' products to other plant sections, especially the roots, and conveying water and nutrients to the leaves. The stem is a component of a plant that is frequently exposed to NPs when NPs are used as soil fertilizers by which NPs enter through the root system (Ali et al. 2019; Abbas et al. 2021a, b). Seed treatments with NPs, such as priming and soaking, may also potentially produce morphological changes in the stem. (Bamsaoud and Bahwirth 2017; Mutlu et al. 2018; Choudhary et al. 2019; Galaktionova et al. 2020; Ramesh et al. 2021). On the other hand, NPs could enter the stem and roots through the leaves system when foliar spraying is used (Haytova 2013; Deshpande et al. 2017; Ali et al. 2019). Therefore, the various effects of NMs on a plant stem need to be recognized since the stem, on the other hand, is believed to be capable of photosynthesis.

Numerous reports in the literature confirm that nano-forms of applied materials positively affect plant stem morphology, mainly stem length, while just a few reports reveal negative consequences (Kasote et al. 2019; Rahman et al. 2020). The most frequent materials in their nano-form are metal and metal compounds. These NMs could affect the hypocotyl and plumule or/and stem diameter (Choudhary et al. 2019; Khan et al. 2020) and/or length as well as other physiological characteristics of the stem. In general, without referring to all the reported nanomaterial-based agriculture treatments, researchers found an increase in plant height, which most likely relates to a change in stem length (Behboudi 2018; Bhatia et al. 2014; Choudhary et al. 2019; Dhoke et al. 2013; Khan et al. 2021; Sharifi et al. 2016; Shinde 2020). However, the ambiguity of claiming that activating various enzymes by some NPs may cause an increase in the length of the plant stem necessitates additional research activity to prove or/and understand the precise reason.

One of the reactions of plants to NPs materials, according to published studies, is an increase in the length of the stem. Dhoke et al. (2013) observed a substantial rise in the stem of mung (*Vigna radiate* L.) when ZnO, nano FeO, and nano-ZnCuFe-oxide particles were applied by foliar spray (Dhoke et al. 2013). Plants treated with Zn Fe Cu oxide NPs had a 15.71% increase in shoot length, whereas plants treated with FeO NPs had a 10.25% increase and ZnO NPs had a 6.47% increase in shoot length compared to control. When a foliar spray of Fe NPs was given to forage maize (*Zea mays* L.), a significant increase in plant height was observed (Saedpanah et al. 2016). Fe NPs enhance plant height by 23% compared to the control, while Zn increases plant height by 5%, respectively. In a different study, the height of forage maize (*Zea mays* L.) was increased by 37% and 24% compared to the control when nano-Fe and nano-Zn were applied individually (Sharifi et al. 2016). Ali et al. (2019) observed an increase in shoot length after foliar spraying wheat (*Triticum aestivum* L.) using Si NPs. Tovar et al. (2020) combined nutrients into iron NPs and applied them to Corn (*Zea mays* L.) seedlings. After 30 days, all samples were identical in terms of stem length compared to the control. The height of maize plants rises by around 20% when Zn NPs are employed as a soil fertilizer and foliar spray, compared to control (Abbas et al. 2021a, b).

Regarding the method of treating seeds with NPs, Bamsaoud and Bahwirth (2017) observed an increase in the length of hypocotyls of *Cucurbita pepo* L. when seeds were treated with silver NPs prepared via *neem* extraction. The highest length of hypocotyls (increased by 12% to control) was noted for seeds treated with silver NPs prepared using Neem. Furthermore, when seeds were immersed in chemically produced silver NPs, the hypocotyls of wheat grains *Triticum aestivum* L. increased by 12%. (Bahwirth and Bamsaoud 2020). Maswada et al. (2018) performed experiments on Sorghum seeds. The seed priming with nano-Fe_2O_3 was more effective than seed soaking in enhancing seedling growth, and the experiments showed that the seedling length increased by 33%. The experiments of Raj and Chandrashekara showed that the higher plant height of Cotton (*Gossypium hirsutum* L.) was around 24% higher than the control gave. In their experiments, the seeds were treated with chelated nano ZnO followed by foliar application of 1000 ppm nano ZnO (Raj and Chandrashekara 2019). Joshi et al. (2021) primed tomato seeds (cv. Sagar) with Selenium NPs by mixing Se NPs solution with 400 tomato seeds. The experiment's findings revealed a substantial increase in plant height (51.2%) for treated tomato seeds compared to control ones. Nematzadeh's studies showed that silver NPs treatment at 80 ppm concentration did not prevent germination at high salinity despite a progressive increase in salinity levels. The stem of *Satureja hortensis* L. increased by 15% compared to the control (Nejatzadeh 2021). Due to the small size, NPs reach the branch through stomates or the base of trichomes in the leaves (Eichert et al. 2008; Uzu et al. 2010). Even though NMs caused a significant difference in stem traits, no research is available and directly studies the considerable reasons for improving plant stem.

12.5 Effect of NPs on Leaves

The leaves consist of stomata or cuticles that allow entering the NPs > 10 nm. Their transfer through the cellular membrane occurs by apoplastic (between 50 and 200 nm) and symplastic (between 10 and 50 nm) routes into the vascular system of the plant (Ali et al. 2021). There are essential factors that affect the existence of NPs on the leaf's surface, such as leaf morphology and its chemical composition, the presence of trichrome, and the existence of leaf exudates (Al-Hadede et al. 2020). The fullerene transmission in the plants is similar to the route of nutrients and water through the xylem (Aslani et al. 2014). In addition, the fullerene existed as black aggregates form in seeds and roots compared to the stems and leaves for rice seeds (Sanborn et al. 2018). The spinach leaves grew better with titanium-oxide NPs through foliar spray (Hossain et al. 2020). The fresh weight of the leaves coffee plant is increased when applied ZnO NPs in percentages of 95% as compared to control (Predoi et al. 2020). The favorable effect of nano-FeO and nano-ZnCuFe oxide was observed on the growth of mung (*Vigna radiate* L.) seedling and leaf and pod dry weight on soybean yield (Asadi-Kavan et al. 2020). Likewise, the number of leaves increased in percentage by 21.42% compared to control plants when applying nano-Fe-EDTA. Magnetic Fe-NPs by Low concentrations increases significantly the chlorophyll contents in sub-apical leaves of soybeans under hydroponic conditions. In contrast, the high amount of iron oxide (Fe_3O_4) as a magnetic nanomaterial harmed plant growth (Predoi et al. 2020). The nano-organic iron chelated fertilizers demonstrated high absorption, increase in photosynthesis, aided in the transfer of iron photosynthate, and expansion in the leaf surface area of peanut plants (Singh et al. 2015).

Agronomic use efficiency for nano-SiO_2 is higher for foliar application than soil application (Predoi et al. 2020). When seedlings were treated with SiO2 NPs, their photosynthetic rate increased. Carbonic anhydrase activity and photosynthetic pigment production both contributed to this rise. (Siddiqui et al. 2020). Using Si-NPs as fertilizer with different concentrations promoted plant height, leaf number, and root length of *Solanum lycopersicum* L. (Predoi et al. 2020). As well as advertised net assimilation rate (NAR), leaf area index (LAI), relative growth rate (RGR), and yield of soybean plants but did not affect height, leaves number, or stem diameters of plants (Siddiqui et al. 2020). It is suggested that the accumulation of Si in leaves is beneficial in maintaining leaves upright and stretching leaf surfaces to capture maximum sunlight, thus optimizing photosynthesis (Predoi et al. 2020). When nano SiO_2 was applied to Changbai larch (*Larix olgensis* Henry) seedlings, it further developed seedling development and chlorophyll biosynthesis (Singh et al. 2015). Adding low concentrations of CeO_2 NP (125 and 250 mg/kg) prompted grain creation, though significant measures of Ce are collected in grains and leaves (Ali et al. 2021).

Al_2O_3 NPs had no significant toxic effects on root elongation and some leaves of Arabidopsis thaliana at concentrations up to 4000 mg/L (Remedios et al. 2012). Using Au NPs resulted in a better crop yield through a favorable influence on the number of leaves, leaf area, plant height, and sugar and chlorophyll content (Singh et al. 2015). On the contrary, during in vitro cultures of Barley, Au NPs supplementation resulted in dark roots, yellow leaves, and decreased biomass (Khana et al. 2019).

12.6 Effect of NPs on Flowers

The flower is a stem bearing leaves that are specialized for sexual reproduction. Many scientists have discussed the effect of NMs on flowers, particularly in terms of increasing their number or their opening speed. Table 12.1 shows the NMs that increased the number of flowers or their opening speed.

12.7 Effect of NPs on Fruits

The researchers used many nanocomposites with different concentrations on different plant species to study their effect on production and increase the yield. Table 12.2 shows the nanocomposites and their concentrations and the extent of the increase in the production of different plant species.

12.8 Conclusion and Prospects

Nanotechnology has excellent potential for multidisciplinary studies in agriculture, including improving the agricultural industry. Despite the research that focuses on realizing the beneficial effects of NPs on plants, it is still incomplete. The effects of NPs differ from one plant to another and is dependent on the technique of production, application, size, shape, and concentrations, according to the data obtained. Also, biological nanocomposites have positive and negative effects that must be studied carefully. For example, soaking seeds into NPs improves germination, growth, and production characteristics. NMs can be exploited to overcome different stresses size, and concentrations of NPs did not show any adverse effects; on the contrary, they showed beneficial currents.

On the other hand, it has been observed that more significant quantities of NPs/NMs are hazardous to plant development, which ultimately depends on particle size. Therefore, in future research, a checkpoint might be established to define the threshold concentration of particular NPs/NMs of a specific size, and the alternative combinations need to be checked. There is a broad scope for green nano-feeding crop plants considering the nanotoxicity effects of NMs/NPs reported. Consequently, green NMs/NPS may be utilized as a source of nutrients for crops and can play an essential part in greener nano feeding for environmental sustainability.

As a future view, promoting the activation of multidisciplinary joint collaborative efforts, combining complementary professional skills such as plant biologists, geneticists, chemists, biochemists, and engineers, may offer new possibilities in phytotechnology. For example, in agriculture, genes have been changed in many plants to improve genetic traits and resistance to diseases and pests. Also, involving

Table 12.1 The effect of different NPs on the flowers of various plants

References	Concentration	Size (nm)	Materials	Effect on plant	Plant species
Marchiol et al. (2016)	500–1000 mg/ kg	25	CeO_2	the reduction in number of spikes/ plant	Barley (*Hordeum vulgare* L.)
Naing et al. (2021)	25 mg/ L	–	Ag	Improvement of flower longevity	Carnation (*Dianthus carphyllus* L.)
Atefepour et al. (2021)	15 mg/ L	20	Ag	shelf-life improved	Gerbera (*Gerbera jamesonii* Bolus ex Hooker f.)
Asgari et al. (2014)	2.5 gr/L	–	Nano-potash (K)	quality improved	Narcissus (*Narcissus tazatta* L.)
Laware and Raskar (2014)	20 –30 mg./mL	18	ZnO	Early flowering	Onion (*Allium cepa* L.)
Prasad et al. (2012)	1000 ppm	25	Zone		Peanut (*Arachis hypogaea* L.)
Reza et al. (2014)	6–12 g	–	Fe, P, and K	Increased Flowers number	Saffron (*Crocus sativus* L.)
Kisan et al. (2015)	500–1000 ppm	50	ZnO	increased leaf length, width, surface area	Spinach (*Spinacia oleracea* L.)
Khodakovskaya et al. (2013)	50–200 µg/mL	10–25	C	Increased number of flowers	Tomato (*Solanum lycopersicom*, L. Mill)

Table 12.2 The effect of different NPs on the fruits of various plants

References	Concentration	Size (nm)	Nanoparticles	Effect on plant	Plant species
Das et al. (2016)	1.6–2.1 mg/g	100–200	FeS_2	Fresh weight (biomass) (g)	Alfalfa (*Medicago sativa* L.)
Kumar et al. (2013)	10 µg/ml	24	Au	Increases yield of fruit/seed	Arabidopsis (*Arabidopsis thaliana* (L.) Heynh)
Salama et al. 2019	30 ppm	–	ZnO	the highest yield of seeds	Bean (*Phaseolus vulgaris* L.)
Das et al. (2016)	1.6–2.1 mg/g	100–200	FeS_2	Increase yield	Beetroot (*Beta vulgaris* L.)
Kole et al. (2013)	–	47.2	$C_{60}(OH)_{20}$	The yield increased by increasing the length, weight and number of fruits	Bitter Melon (*Momordica charantia* L.)
Arora et al. (2012)	10 ppm	300–600	Au	increase in seed yield	Brassica (*Brassica juncea* L.)
Das et al. (2016)	1.6–2.1 mg/g	100–200	FeS_2	Increase yield	Carrot (*Daucus carota* L.)
Mahajan et al. (2011)	1 ppm	20	ZnO	Increased yield	Chickpea (*Cicer arietinum* L.)
Merghany et al. (2019)	6 ml	9.165, 8.254 and 8.205 (N, P and k)	Liquid nano NPK		Cucumber (*Cucumis sativus* L.)
Sabet and Mortazaeinezhad (2018)	0.5–1 g/L	–	Fe–N	Increased yield and weight of 1000 grains	Cumin (*Cuminum cyminum* L.)

(continued)

Table 12.2 (continued)

References	Concentration	Size (nm)	Nanoparticles	Effect on plant	Plant species
Raliya and Tarafdar (2013)	10 ppm	1.2–6.8	ZnO	Increases biomass	Custer bean (*Cyamopsis tetragonoloba* L)
Younes et al. (2019)	0.1, 0.2, and 0.3 g/L	–	Graphene	Increases number of fruits/plant and fruit yield (ton/hectare)	Eggplant, (*Solanum Melongena* L)
Abbas et al. (2021a, b)	0.0015 g/ L	30	ZnO	Increases grain yield and 100-grain weight	Maize (*Zea mays* L.)
Mahajan et al. (2011)	20 ppm	20	ZnO	Increased yield	Mung Bean (*Vigna radiata* L.)
Ibrahim and Al Farttoosi (2019)	0.009 g/L	–	B		
Das et al. (2016)	1.6–2.1 mg/g	100–200	FeS_2	increase in the seed yield	Mustard (*Brassica juncea* (L.) Czern
Rahman et al. (2020)	1 mM	3.2 ± 0.8	Pt	average number of seeds produced per plant increased	Pea (*Pisum sativum* L.)
Tarafdar et al. (2014)	0.1 mM	15–25	Zn	Improved The grain yield	Pearl Millet (*Pennisetum americanum* L.)
Prasad et al. (2012)	0.13 g /L	25	ZnO	Increased pod yield	Peanut (*Arachis hypogaea* L.)
Liu et al. (2005)	0.5 g/L	–	CaCO	The highest yield	
Younes et al. (2019)	0.1, 0.2, and 0.3 g/L	–	Graphene	Increases number of fruits/plant and fruit yield (ton/hectare)	Pepper, (*Capsicum annuum* L.)

(continued)

Table 12.2 (continued)

References	Concentration	Size (nm)	Nanoparticles	Effect on plant	Plant species
Sohrab et al. (2016)	636 mg/ tree and 34 mg /tree respectively	50	Zn and B	Increased number of fruits per tree, and fruit yield	Pomegranate (*Punica Granatum* L.)
Tahmasbi et al. (2011)	0.05 g/L	–	Ag	Produced higher yield	Potato *Solanum tuberosum subsp. andigenum* (Juz. & Bukasov) Hawkes
Mahmoud Abdel Wahab et al. (2019)	60 and 50 ppm, respectively	410	FeO-ZnO	Increase root weight and diameter	Red radish (*Raphanus sativus* L.)
Liu et al (2009)	5 mM	4–10	SiO2	Increased grain weight	Rice (*Oryza sativa* L.)
Anusuya et al. (2019)	0.5% $ZnSO_4$	–	Zn	Increased productivity	
Seleiman et al. (2020)	-	18	ZnO	Increase yield	
Das et al. (2016)	1.6–2.1 mg/g	100–200	FeS_2	Increase in the number of pods and seeds	Sesamum (*Sesamum indicum* L.)
Sheykhbaglou et al. (2010)	0.5 g/L	–	Fe_2O_3	Increased grain yield	Soybean (*Glycine soja Sieb.* Et Zucc.)
Khodakovskaya et al. (2013)	0.05–0.2 g/L	25	C	Improved fruit yield	Tomato (*Solanum lycopersicom*, L. Mill)
Asma et al. (2019)	0.088 mg/L	–	Ag	Increases yield/plant (g),	
Younes and Nassef (2016)	10, 20, 40 ppm	–	Ag	reduced the fruit number per plant, fruit diameter, average fruit weight,	
Razzaq et al. (2016)	25 ppm	10–20	Ag	Increasing grain number /spike and crop yield	Wheat (*Triticum aestivum* L)

(continued)

Table 12.2 (continued)

References	Concentration	Size (nm)	Nanoparticles	Effect on plant	Plant species
Shafaqat et al. (2019)	–	≤50	Si s	Increases the yield and reduce the Cd in the grains	
Behboudi et al. (2018)	30–60 ppm	40 ± 9.5	SiO_2	Increased Final yield	
Rico et al. (2014)	500 mg/kg	8 ± 1 (rod) and 231 ± 16 (particle)	CeO_2	Improved grain yield	
Hafeez et al. (2015)	30 ppm	12- 20	Cu	Increase grain yield	
Bakhtiari et al. (2015)	0.04% w/v	–	Fe_2O_3		

the plant extracts in synthesizing NPs is the safest material for agriculture that remains an open framework with promising results.

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Chapter 13
Role of Nanomaterials in Improving Crop Productivity

Lina M. Alnaddaf, Jameel M. Al-Khayri, and S. Mohan Jain

Abstract Nanotechnology is currently used in various fields due to the unique properties of nanoparticles (NPs). These properties are important in agriculture for their effect on plant growth and development (stimulating or inhibiting). As well as, obtain the best productivity in quantity and quality. The effect of NPs varies according to plant species, NPs and their different properties (size, shape, concentration, surface and other characterization). NPs accumulate in different plant parts which are reflected as well positively or negatively, quantitatively and qualitatively in plant growth and development. The nutritional value of treated plants is enhanced as a result of increasing the nutrient concentration which is reflected positively in human and animal nutrition. Similarly, the NPs positively promote the growth and development of plants, leading to an increase in the yield and productivity of plants. Whereas, this accumulation may have phytotoxicity effects on the plant and the accumulation of free radicals and abnormal cell division decreases plant production. Therefore, more research is needed to investigate the effectiveness of different particles on various plant species.

Keywords Crop productivity · Crops quantity · Crops quality · Nanoparticles characterization · Nanoparticles · Role of nanomaterials

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13.1 Introduction

Nanotechnology will be the best option in the agricultural sector through its applications in several fields in a sustainable manner (Shahrajabian et al. 2021). For example, a lot of yield seeds and fruits will be natural throughout the year. Likewise, helps in the protection of plants through the early detection of diseases and stresses. Also, nanostructured catalysts are accessible to raise the effectiveness of pesticides and herbicides. It is necessary to comprehend the activity and impacts of nanoparticles (NPs) when being applied to crops (Rivero-Montejo et al. 2021).

The surface structure, shape, chemical composition, size, concentration, solubility, and aggregation of engineered nanomaterials (NM) are fundamental qualities to understanding their impact on plants. Additionally, plant species have various responses (enhancing or inhibiting) to different NPs (Fig. 13.1). The impact of engineered NM on plants can change with the phases of plant development and period of exposure (Abd El-Moneim et al. 2021). NM promotes various plant essential parameters upon absorption via roots and leaves which are the vitally supplement entree of plants (Shang et al. 2019). All changes caused by the NPs in plant physiological parameters, morphological traits, and gene expressions are vital to further enhance field yields to satisfy future food needs. Understanding both the positive and negative impacts of NPs is important to realize higher crop productivity. Despite what might be positively expected, hazards must be identified with a long exposure of farmers to NM, various environmental interactions, and conceivable intensified bioaccumulation of NPs, all of these aspects ought to be taken into account before nanotechnology application (Abd El-Moneim et al. 2021).

This chapter describes various properties of NM and their effects on crop production and the importance of nanotechnology in agriculture. Furthermore, it highlights features of NPs as well as the differences between nano fertilizers (NFs) and conventional fertilizers including the factors that affect their properties, and the impact of NPs on crop production quality and quantity.

13.2 Features of NM to Improve Crop Productivity

Nanotechnology further impacts on crop yields and uses NPs effectiveness to lessen extreme wastage from the fertilizers utilized (Mali et al. 2020). Thirty-three percent of crop productivity is dependent on fertilizers and the utilization effective of other agricultural inputs. Nevertheless, the benefit from using conventional fertilizers does not exceed 30–40%.

The plants benefit from small amounts of traditional fertilizers utilized, and the bulk remains in the soil, leading to environmental pollution, the negative impact of which reflects on the typical development of flora and fauna (Shang et al. 2019). In addition, the plant's benefit from the added fertilizers, whether adding to the soil or

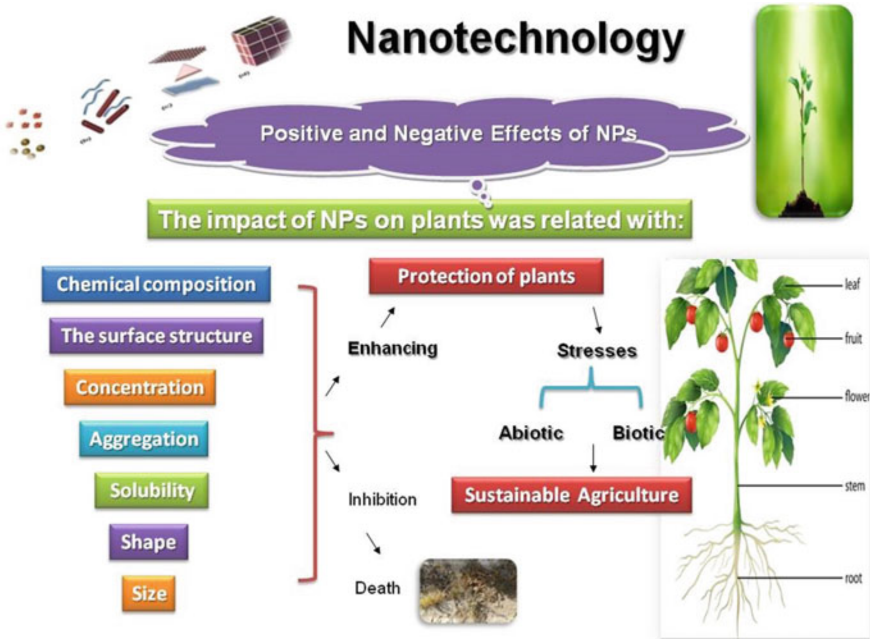

Fig. 13.1 The influence of nanotechnology on sustainable agriculture. Plants have various reactions (enhancing or inhibiting) related to NPs properties, different growth stages and conditions (Constructed by L .M. Alnaddaf)

sprayed on the leaves, is related to the concentration used, which reaches the target plant part (Solanki et al. 2015).

Plant absorbs a very small amount of it. In contrast, a large amount of fertilizers is lost by runoff, evaporation, drift, photolytic, hydrolysis, and microbial degradation leading to salts aggregation and soil deterioration (Sabir et al. 2014). Therefore, NFs introduce the solutions for what was previously mentioned. Appropriately, nano formulations inhibit the losses of macronutrients and micronutrients via plant absorption of the added quantities needed by the plant as a result of the properties of NM, by shaping a nano network in the soil via surface tension and molecular force. The network keeps nutrients around plant roots, to soak up the needs all through the development plant cycle (Shang et al. 2019). Additionally, the size decrease of NM leads to an increase in the surface mass proportion of particles; accordingly, plants absorb these nutrient particles and dissolve them gradually for a lengthy time frame (Monreal et al. 2016) (Table 13.1). An assortment of materials is utilized to create and cover NM; for example, plant extracts, polymers, metal oxides, lipids, ceramics, and emulsions (Mali et al. 2020).

Various research showed that nano calcite $CaCO_3$ (40%) application with nano SiO_2 (4%), Fe_2O_3 (1%), and MgO (1%) not only enhanced the take-up of Ca, Mg, and Fe but improved the sucking of P, micronutrients Zn and Mn prominently (Sabir et al. 2014). NFs deliver their essential nutrients in 40–50 days, while traditional

Table 13.1 Comparison between the use of conventional and nanofertilizer in agriculture

Fertilizers properties	Conventional fertilizers	Nano fertilizers
Utilization amount from fertilizers	Large	Small
Utilization efficiency from fertilizers	Low	High
Benefit from fertilizers %	30–40%	60–70%
Plant absorption	4–10 days	40–50 days
Environmental pollution	Yes	Less pollution

fertilizers do likewise in 4–10 days. Therefore, over 70% of traditional urea fertilizer added to field, is quickly lost through filtering and volatilization and leaving about 20% easily accessible for plants (Seleiman et al. 2021).

One of the main highlights of NFs is small particle size, which gives it the ability to enter plants when it is utilized as soil amendments or spray of leaves. Besides, NFs are different from artificial fertilizers by the ability to solve in water and evenly distributed in the soil solution. In addition, NFs give extreme reactivity and constant availability of nutrients to plants as a result of having high surface areas (Liscano et al. 2000; Siddiqi and Husen 2017). As of late, NFs were prepared by covering the area with hydroxyapatite NPs, which slowed the absorbance of N as much as 12 times slower than traditional urea in the rice (*Oryza sativa* L.). This led to increased grain yields at only 50% of the rate compared with traditional urea (Seleiman et al. 2021). Likewise, utilizing a zero-urea nano fertilizer on maize plants produces better grain quality, growth, and yield development compared with conventional usage (Manikandan and Subramanian 2016).

The utilization of chitosan and zeolites has contributed to significantly further developing absorb efficiency by controlling the release and reducing the amount of Nitrogen (Abdel-Aziz et al. 2016). For instance, using urea-modified zeolites increased the seed yield of soybean (*Glycine max* L.) (Liu and Lal 2015), (*Solanum tuberosum* L.) (Zareabyaneh and Bayatvarkeshi 2015) and (*Brassica napus* L.) (DeRosa et al. 2010; Liu and Lal 2015).

Likewise, nano-formulations of Phosphor (p) decrease the loss of P through the utilization of ammonium zeolites and absorb it immediately by the plant (Dwivedi et al. 2016). Therefore, the utilization of P-enriched hydroxyapatite NPS in soybeans (Glycine max L.) increases the grain yield (18%), shoot growth, and plant height contrasted with p fertilizer (Liu and Lal 2015; Shalaby et al. 2016). In addition, nano capsules are used as smart delivery for herbicides and pests that have less effect on human health and the environment. Adding macronutrients, micronutrients like manganese, copper, boron, chlorine, iron, zinc, and molybdenum assume a fundamental part in developing and increasing crop productivity (Shang et al. 2019).

13.3 Impact of NPs on Crop Quality

NFs provide crops with a balanced nutrient throughout the growth cycle that ultimately improves agricultural production. It should be noted that increased productivity might encourage farmers to use the product more efficiently (Rajputa et al. 2020) (Table 13.2). As a promising field of multidisciplinary research, nanotechnology has revived its greatness in agriculture. These changes will bring benefits to feeding malnourished people and feeding animals with rich, nutritious grass. The absorption of NPs varies with the species of plant, tissue/organ utilized directly for food or food processing, and the type and size of NPs (Elshayb et al. 2021). This may lead to the accumulation NPs in various tissues and cause toxic problems in both plants and humans (Zulfiqar et al. 2019).

In red spinach (*Amaranthus tricolor* L.) multi-walled carbon nanotubes had phytotoxicity effects resulting in growth inhibition, cell death, and producing reactive oxygen species (Pullagurala et al. 2018). CeO_2 NPs increased the nitrogen potential percentage of soybean causing human health problems (Priester et al. 2012). In addition, apply of fullerene increased the accumulation of dichlorodiphenyldichloroethylene (DDT) in zucchini, soybean, and tomato plants (Torre-Roche et al. 2012).

Rico et al. (2013) planted three varieties of rice with a variety of amylose grains (low, medium, and high) in the soil with 500 mg CeO_2NPs/kg soil. CeO_2 NP treatment grains contained small amounts of starch, prolamin, glutelin, iron, sulfur, valeric, and lauric acid. NPs treatment lower antioxidant levels, in addition to those flavonoids, in grain. The concentration of Ce in the grain was greater in low- and medium-amylose cultivars than in high-amylose cultivars (Khan et al. 2020).

ZnNPs promote the quality and yield of coffee beans by having a positive effect on color index, polyphenol oxidase activity, caffeine and trigonelline, chlorogenic acid, and sucrose.

Nano-Ca fertilization decreases the total number of phenolic compounds in pomegranate fruit juice and has no significant effect on overall anthocyanin content, antioxidant activity, and the harvest of cherries, strawberries, grapes, and kiwifruit (Predoi et al. 2020).

13.4 Impact of NPs on Crop Quantity

The plants demonstrated different responses in the plant growth and development resulting from NPs treatment (Table 13.3). Seed treatment of spinach (*Spinacia oleracea* L.) by TiO_2 NPs (0.25–4.0% w/v) has a positive effect on biomass accumulation (60%), increasing some indicators such as plant dry weight (73%), chlorophyll (45%), photosynthesis rate rubisco activity and nitrogen assimilation. This positive effect is related to decreased TiO_2 particle size (30–40 nm) (Masarovičová and Kráľová 2013).

Table 13.2 Various effects of NPs on **the quality** indicators of different plants

Plant species	Nanoparticles	Effects	References
Apium graveolens L	Se	Increased of total phenols and flavonoids, soluble sugar, beta-carotene, tryptophan, chlorophyll, proteins, proline, glutamic acid, arginine, jasmonic acid, aspartic acid, total antioxidant capacity and vitamin C	Li et al. (2020a, b)
Bacopa monnieci L	Ag NPs	Increased The germination rate, the proteins and carbohydrates content. Decreased catalase activity, the phenol content, and peroxidase activity	Khan et al. (2020)
Calendula officinalis L	(methyl jasmonat) MeJA and Ag	Promote saponin. Rising membrane lipid peroxidation. Whereas, flavonoid, anthocyanin, carotenoid, chlorophyll content, DPPH radical scavenging activity were decreased	Ghanati and Bakhtiarian (2014)
Camelina sativa L	ZnO	Rise of anthocyanins, carotenoid total phenol content, phosphorus, calcium and zinc. Decreased total flavonoid content and antioxidant capacity	Hezaveh et al. (2020)
Capsicum annuum L	Cu absorbed on chitosan hydrogel	Maintaining post-harvest weight fruits. The content of phenols and flavonoids, antioxidants and titratable acidity was raised	Pinedo-Guerrero et al. (2017)

(continued)

The spray of nano-iron oxide (0.5–0.75 g/dm^3) increased the grain yield of soybean by 48%. In addition, it had a positive impact on the dry weight of a leaf and pod. Likewise, TiO_2 and SiO_2 enhanced seedling growth and germination of soybean (Sheykhbaglou et al. 2010). In addition, using nano-sized hydroxyapatite (nHA) improved seed yield (20.4%) (Seleiman et al. 2021).

Table 13.2 (continued)

Plant species	Nanoparticles	Effects	References
Catharanthus roseus L	Fe_3O_4 NPs	Increase in leaf growth parameters and the carbohydrate contents. The quantity of antioxidants, proline, and density of leaf stomata was not influenced	Seleiman et al. (2021)
Corylus avellana L. cv. Gerd Eshkevar	Ag	Rising taxanes content (taxol and baccatin III) and lipid peroxidation. Reducing total contents of flavonoids and soluble phenols	Jamshidi and Ghanati (2017)
Cucumis anguria L	AgNPs and $AgNO_3$	Increased the biomass accumulation, total phenols content and biological activity (antibacterial, antioxidant, anticancer and antifungal)	Chung et al. (2018)
Cucumis sativus L	Cu	Rising most of the organic compounds such as leucine, valine, isoleucine, threonine, tyrosine, fructose, xylose, glutaric acid, benzoic acid, linolenic acid, caprylic acid, glycine, imidazole and proline. whereas decreased methionine and lysine	Zhao et al. (2017)
Momordica charantia L	ZnO NPs, jasmonate and chittosan	Increase anthocyanin content, flavonoids, phenols, carbohydrate as well as carotenoids. Antioxidant enzyme activity and proline content too	Sharifi-Rad et al. (2020)
Prunella vulgaris L	Ag, Au and naphthalene acetic acid (NAA)	Promoted total phenols and flavonoids, biomass, and DPPH-radical scavenging activity	Fazal et al. (2019)

(continued)

Table 13.2 (continued)

Plant species	Nanoparticles	Effects	References
Solanum lycopersicum L	Cerium oxide ($nCeO_2$) and Citric acid coated cerium oxide ($nCeO_2$ + CA)	($nCeO_2$) Increased of lycopene. Whereas, $nCeO_2$ + CA reducing sugar and starch	Barrios et al. (2017)
	Cu absorbed on chitosan hydrogel	Increased activity of titratable acidity, catalase activity and lycopene	Juarez-Maldonado et al. (2016)
Stevia rebaudiana (Bertoni) Hemsl	ZnO and CuO	Increased total phenolic content, total flavonoid content, total antioxidant activity. Reduced weight and quality of callus at high concentration	Ahmad et al. (2020)
Tanacetum parthenium L	SiO_2 and TiO_2 NPs	Rising of TpGAS, COST, TpCarS gene and β-caryophyllen biosynthesis pathway	Khajavi et al. (2019)
Withania somnifera L. Dunal	CuO	Polyphenols and antioxidant activity were influenced	Singh et al. (2018)

The application of carbon NPs with fertilizer promoted grain yields of soybean (16.74%), spring maize (10.93%), rice (10.29%), and vegetables (12.34–19.76%) and winter wheat (28.81%) (Shang et al. 2019). Nano-K fertilizer-loaded zeolites affect the K concentration, harvest index, yield, and chlorophyll content of hot pepper (*Capsicum annuum* L.) (Seleiman et al. 2021). In addition, the same influence was observed in the biomass, growth, and quality of *Cucurbita pepo* L. (Gerdini 2016). Au NPs (10 ppm) led to promote seed yield and growth in mustard (Khan et al. 2019).

Furthermore, developed seed germination affected the expression of miRNA in *Arabidopsis thaliana* L. which led to the development of growth and yield (Kumar et al. 2013). There were positive effects in *Quercus macdougallii*. Martínez. a result of the Fe_2O_3 NPs treatment such as Plant growth, seed germination, chlorophyll content, and biomass (Pariona et al. 2017). The foliar application of Zn, Mg, and Ti NPs had an effective impact on the growth and yield indicators for watermelon (Wang et al. 2016).

Rico et al. (2015) indicated that using CeO_2 NPs increases plant biomass in barley. Also, zinc oxide NPs enhance physiological responses, shoot and root elongation, photosynthesis, fresh, dry weight, stimulate nutrient use efficiency and raise productivity by way of ninety-one%, while conventional bulk $ZnSO_4$ increases productivity by 31% as compared to the control (Kale and Gawade 2016). The same influence on zinc oxide NPs was observed for peanuts (Elemike et al. 2019).

Table 13.3 Various impact of nanotechnology on the quantity indicators of Crops

Plant species	Nanoparticles	Effects	References
Arabidopsis thaliana L	Fullerenes	Enhanced yield	Gao et al. (2011)
Arachis hypogaea L	Nano-potash (K)	Enhanced yield	Asgari et al. (2018)
	Ag	Reduced yield and plant growth parameters	Rui et al. (2017)
Cicer arietinum L	FeS_2	Enhanced yield	Das et al. (2016)
Cuminum cyminum L	Nano-iron oxide (Fe)	Promoted stem length, Fe concentration and yield	Sabet and Mortazaeinezhad (2018)
Cyamopsis tetragonoloba L. Taub	ZnO NPs	Increased nutrient contents, biological yield and growth	Raliya and Tarafdar (2013)
Glycine max L. Merr	ZnO, B_2O_3, CuO	Increased dry weight, count, the content of N and K of grain	Dimkpa et al. (2017b)
Oryza sativa L	CuO	Grain yield and Physiological parameters adversely influenced. the grain content of Cu and Zn greatly existed in mature plants	Peng et al. (2017)
	TiO_2	Decreased grain yield and plant biomass	Du et al. (2017)
Pennisetum americanum (L.) Leeke	Zn NPs	Enhanced yield	Zulfiqar et al. (2019)
Pisum sativum L	Fe-based NFs	Rising seed weight and chlorophyll contents	Giorgetti et al. (2019)
Solanum lycopersicum L	Cu NPs	Rising fruit firmness and antioxidant contents	Ahmed et al. (2018)
	Ag with PEG coating	Reduced chlorophyll content, NPK uptake, fruit yield; increased fruit Ag content	Das et al. (2018)
	MWCNTs	Enhanced yield	Khodakovskaya et al. (2013)
Sorghum bicolor L. Moench	ZnO	Increased grain content of Zn, N, K, and P and grain yield	Dimkpa et al. (2017a)
Spinacia oleracea L	TiO_2	Increased nitrogen and protein content, chlorophyll, biomass accumulation	Yang et al. (2007)

(continued)

Table 13.3 (continued)

Plant species	Nanoparticles	Effects	References
Triticum aestivum L	ZnO NPs	Rising biomass accumulation and grain yield	Du et al. (2019)
	CuO NPs	Enhanced yield	Dimkpa et al. (2012)
	Chitosan-NPK	Decreased protein content. Whereas increased P and K contents	Abdel-Aziz et al. (2018)
	Nano-silica (SiO_2)	Enhanced yield	Behboudi et al. (2018)
	MWCNTs	Enhanced yield	Wang et al. (2012)
	ZnO	Increased in Grain yield and Zn content	Dimkpa et al. (2018)
Zea mays L	MWCNTs	Rising nutrient transport and yield	Tiwari et al. (2014)
	ZnO NPs	Increased morphological parameters, chlorophyll content, and grain quality	Raliya et al. (2016)

The exploitation of the solar spectrum via CuInS2/ZnS quantum dot (QD) films improves the absorption of blue photons and redshift ultraviolet, which led to an increase in the biomass of red romaine lettuce (Parrish et al. 2021). Cu NPs 600 mg/kg enhance the growth of lettuce seedlings by up to 91% (Predoi et al. 2020).

Comparing the effect of NPs (CuO, ZnO, MnO, and FeO) and ions (Cu, Zn, Mn, and Fe) on the growth of lettuce seedlings *Lactuca sativa* L. with concentrations of (<50 mg/L). The results indicated that toxic effects for CuO NPs were more as compared to Cu ions. While the toxic effect of ions Zn was like that of ZnO NPs. Whereas, MnO and FeO NPs promoted the seedling's lettuce growth from 12 to 54%. In addition, it's less toxic compared with its ionic shapes (Lü et al. 2016).

Multiple wall carbon nanotubes (MWCNT) contribute to stimulating the growth and division of cell tobacco culture (Khodakovskaya et al. 2012). Tobacco seed treatment with TiO_2 NP did not affect its positive growth and development. Using TiO_2 NPs led to reducing the fresh and dry weight of plants and decreased growth and development of roots and shoots (Burklew et al. 2012).

TiO_2 NPs improved photosynthesis by 30% and decreased cucumber infecting with *Psilocybe cubensis* (Earle) by 91%. Also, ZnO NPs (100 mg/kg) reduced the indicator of cucumber growth (Cui et al. 2009). Tomato roots treatment with MWCNT (50 μg/ml) stimulated root biomass. In addition, using Fe_2O_3 NPs as root and foliar applications could promote root elongation and photosynthesis (Predoi et al. 2020).

The effect of silver NPs on a wheat plant varies between positive and negative effects, according to the size of the particles. The positive effect resulting in increased content of aminocyclopropane-1-carboxylic acid (ACC) and phytohormones led to the promotion of growth and production indications, especially when using low concentrations from AgNPs such as 25 ppm and size particles about (50–70) nm. The high concentration of AgNPs and small particle size (30) nm had inhibition effects on wheat growth due to abnormal cell division and toxic effects (Wang et al. 2020).

Apply of TiO_2 NPs at 20 g/L led to promote biomass, ear mass, stem elongation, flowering, and seed number (Predoi et al. 2020). In addition, 2.5% (w/v) TiO_2 NPs contributed to increase seed germination (Khan et al. 2020). Mn NPs improved the growth and development traits of wheat compared with the control; however, increasing the amount of Mn NPs was not useful resulting in competition in transport, uptake, and use with other ions Mg, Fe and Ca (Predoi et al. 2020).

Chitosan-NPK fertilizer enhanced the yield at the rate of 5.5% as well as the crop and harvest indexes compared with the control (Shang et al. 2019). ZnO NPs positively promoted coffee plant biomass, photosynthesis, and the carbon absorption rate and conduct of stomatal which raised the content of CO_2 in the chloroplast and increased rubisco enzyme effectiveness (Predoi et al. 2020).

Applying NPK NF stimulated photosynthesis at 71.7% and the number of leaves. In addition, it increased the NPK contents in a plant by 67.5% compared with the control (Seleiman et al. 2021).

Apply boron (B) (34 mg/tree) and Zn (636 mg/tree) NFs as a foliar spray had positive effects on fruit yield by 30% compared with the control. Also, these treatments affected affirmatively the physical parameters of fruit size such as fruit calyx diameter, fruit diameter, and average weight. There were no significant differences between the treatments and control in pomegranate trees (*Punica granatum* cv. Ardestani) (Predoi et al. 2020). The utilizing foliar spray of boron (B) and Zn NFs for *Dracocephalum moldavica* L., led to improvement the contents of the essential oil beside to stimulate growth and development of it. All of these effects could be clarified by increasing water and nutrient absorption, which promoted the activity of enzymes and root growth (Shang et al. 2019).

Ocimum basilicum L. plants were stimulated by a foliar spray of potassium, calcium, and Fe_3O_4 NFs which affected grain yield, harvest index, and biological yield. In addition, they increased the biochemical indicators such as chlorophyll, carbohydrate, oil levels, and the percentage content of potassium, calcium, and iron (Rautela et al. 2021).

In rice, production improved through enhancement of disease-resistant, resulting in a 14.8% increased yield by using Carbon Dots CDs (0.56 mg/ml) (Li et al. 2020a, b). ZnO NPs were biosynthesized by seaweed *Turbinaria ornata.* (Turner) J. Agardh. promote seed germination, growth and development parameters, 1000 grain weight, and yield for rice plants (Itroutwar et al. 2020). The various effects of nanotechnology during the different stages of plant growth and development are shown in Fig. 13.2.

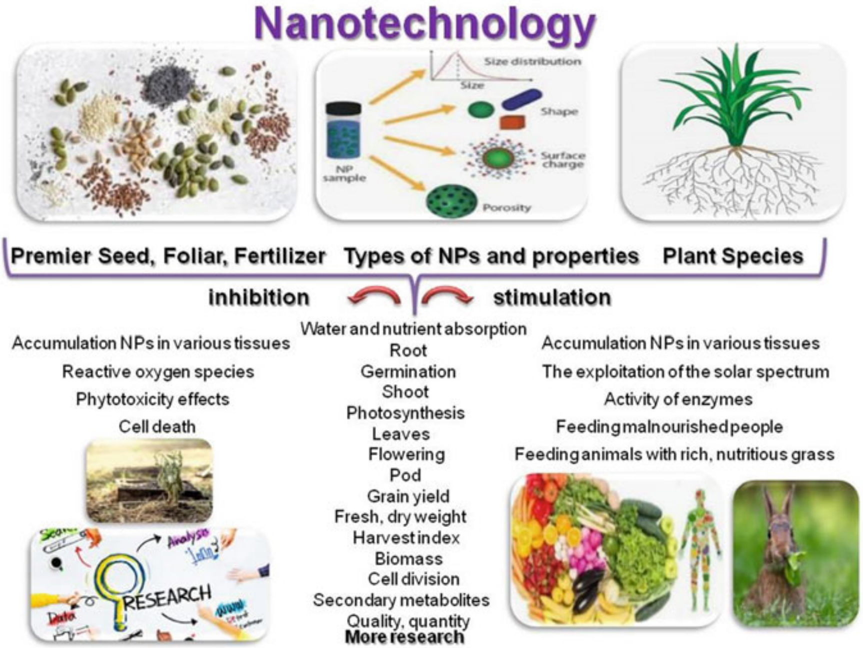

Fig. 13.2 The Inhibitory and stimulatory effects of NPs in plant cells during the different stages of plant growth and development. Also, its cumulative effects in different organisms (Constructed by L. M. Alnaddaf)

13.5 Conclusion and Prospects

Nanotechnology is an advanced tool for sustainable agriculture and increases plant produced in an eco-accommodating way even under various stresses. NPs consider a great promise tool in increasing crop production. Agriculture takes advantage of NM for promoting the productivity of crops. In addition, NFs are eco-friendly, reduce the over-use of conventional fertilizer and are effective for a long time. NPs have toxic effects and causes harm to human, plant, and the environment. NPs interact with various biomacromolecules present in the living system and environments. As well as NPs accumulate in the soil–plant systems. For this reason, the inhibitory or stimulatory effects of NPs in plant cells need more research to illustrate different plant responses in physio-biochemical mechanisms besides ecological, phenological, cytological, anatomical, and molecular mechanisms. Finally, it is essential to balance the applications of NM based on their characterization for the best use in crop production, the reduction of phytotoxicity and increasing the quantity and nutritional value of crops for ensuring food for the increasing world population.

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Chapter 14
Role of Nanomaterials in Plant Cell and Tissue Culture

Sanaz Feizi

Abstract The plant tissue culture (PTC) technique has been established based on totipotency and regeneration capacity of plant cells by culturing different types of explants on a nutritional culture medium for regenerating the whole organ. It has an economically important place and its use in basic sciences such as genetics, biochemistry, tissue engineering, and biotechnology shows its value. This technique may provide some key applications including plant conservation, higher mass reproduction, genetic manipulation, and biologically active compound production. Nanoparticles (NPs) are small particles with a diameter of 1–100 nm. It is recently believed that many nanoparticles NPs could implicate significant effects on the various aspects of plant tissue culture including somatic embryogenesis, organogenesis, callus induction, sacral modification, genetic transformation, control of microbial pollutants, and the production of secondary metabolites. This chapter has focused on the different effects of several important NPs including metal and metal oxide, polymeric, dendrimers, quantum on the various plant abiotic stresses and then a comprehensive application of them on the amelioration of plant growth, crop production, and cytotoxicity remediation and the mechanism of nanoparticles affecting callus and secondary metabolism would be discussed. Of note, we would highlight different approaches to explore appropriate NPS for the improvement of the potential adaptation of plants under abiotic stresses aiming for their sustainable productivity.

Keywords Callus · Nanoparticles · Organogenesis · Plant tissue culture · Secondary metabolites · Somatic embryogenesis

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14.1 Introduction

Plant tissue culture (PTC) is a vital, eco-friendly, and cost-effective technique implicated in different aspects of plant biology such as cell biology, biotechnology, biochemistry, and genetics (Thorpe 2007). This approach may be utilized for the mass propagation of plant cells, the production of genetically modified and free-disease tissues, and the efficient production of secondary metabolites (Khosroushahi et al. 2006). Moreover, PTC may minimize environmental variations by the use of specific and unique nutrient media in a controlled culture condition, nutrient availability in a homogenous manner, and decreased stress severity (Sakthivelu et al. 2008). Tissue cultures such as cell suspensions, callus, and hairy roots provided several advantages including simple and fast exploration of the effects of microflora and also membrane barriers on the cell and tissues compared with the whole-plant systems (Doran 2009). NPs particle size range is from 1–100 nm which provided a much larger surface area to volume ratio resulting in the enhancement of catalytic reactivity, thermal conductivity, biological activity, and chemical steadiness compared to their bulk forms. Accordingly, NPs could be used in health, cosmetic industries, food supplements, agriculture, electronics, and textile industries (Agarwal et al. 2017; Prasad et al. 2017; Dimkpa and Bindraban 2018). Interestingly, several reports identified the positive effects of nanoparticles (NPs) on the plant cells and tissue cultures in which they might significantly increase the secondary metabolite production, induce callus formation, somatic embryogenesis, organogenesis, and facilitate some genetic modifications (Kim et al. 2013). Moreover, a supplement of NPs can effectively lead to the control of microbial pollutants in plant culture medium (Helaly et al. 2014). Further reports confirmed NPs might facilitate genetic engineering procedures during callus regeneration experiments. NPs such as magnetic-related NPs and carbon nanotubes can mediate the accurate transfer of DNA molecules into the cells by reducing the integrity of plant cell walls (Lv et al. 2020). Ag NPs and Au NPs can induce random changes in the coding sequences of pectin methylesterase enzyme and Mlo-like protein during differentiation of callus of Flaxseed (*Linum usitatissimum.* However, the mechanisms of variations in nuclear genome induced by NPs have still been remain ununderstood (Kokina et al. 2017b).

14.2 Impact of Nanomaterials on Callus Induction

Overall, nanomaterials (NMs) have been categorized into Carbon- and metal-related nanomaterials. Carbon-related NMs have included fullerenes, graphene, and carbon nanotubes (e.g., single-walled carbon nanotubes and multi-walled carbon nanotubes) (Buzea and Pacheco 2017). While metal-based NPs are composed of zero-valent metals (e.g., Au, Ag, and Fe), metal oxides (i.e., nano-CuO, -ZnO, -CeO2, -TiO2, -Fe2O3, and -SiO2), quantum dots (CdSe and CdTe), nano-sized polymers (dendrimers and polystyrene), and metal salts (nano silicates and ceramics)

(Dallavalle et al. 2015). Different reports confirmed that the NPs could significantly improve seed germination and bioactive compound production, enhance plant growth and yield, and intensely increase plant protection capacity (Wang et al. 2016; Ruttkay-Nedecky et al. 2017). Although several metallic NPs are currently utilized in the agriculture industry, the release of these molecules into the environment might impose negative cytotoxic impacts on the living organisms. These toxic effects came from the size, morphology, nature, surface area ratio, composition, and reactivity charcharis of metal-based NPs (Zaka et al. 2016). It is frequently reported that metallic stress (Cu, Cd, Al, Pb, and Ni) could stimulate the phenylalanine ammonia-lyase (PAL) and chalcone synthase enzymes resulting in the induction of plant secondary metabolite production (Singh et al. 2015). It seems the higher tendency of phenolic compounds to the chelate metals are involved in the enhanced biosynthesis of these molecules (Jun et al. 2003). In this context, engineered NPs provided some unique physicochemical properties which facilitated their penetration into plant cells and tissues and subsequent delocalization (Keller et al. 2013). Notably, NPs such as gold, cerium oxide, aluminum oxide, and zero-valent iron might increase plant growth rates, modulate gene expression levels, and induce the synthesis of proteins and other metabolites in the different plant cells and tissues (Jaskulak et al. 2019; Montes et al. 2017; Kim et al. 2014; Kumar et al. 2013; Lee et al. 2010; Yang et al. 2017). The effects of several NPs on physiology, morphology and metabolism-related pathways of plant callus or cell suspension cultures obtained from some recent research are discussed below.

Zinc Oxide (ZnO)

Several studies have been performed to evaluate the impact of biosynthesized zinc oxide NPs on in vitro production of bioactive compounds and the improvement of biomass in different plants. It is reported that low concentrations of ZnO NPs could stimulate callus growth and also enhance regeneration, organogenesis, and decontamination (Mousavi Kouhi and Lahouti 2018; Kavianifar et al. 2018). Upon exposure of plant cells to the ZnO NPs, the production of secondary metabolites has been induced in which they functioned as phytoalexins to protect plant cells and tissues against biotic and abiotic stress (Marslin et al. 2017; Abdel-Lateif et al. 2012). Of note, Zinc Oxide NPs might modulate the antioxidant and macromolecules systems in the callus of *Solanum nigrum*. It is identified that the dry weight of callus was increased upon exposure to the lowest concentration of ZnO NPs. Moreover, the activity of lipoxygenase and antioxidant enzymes were increased at the highest level of ZnO NPs. Although the activity of phenolic and phenylalanine ammonia-lyase compounds was not changed by the treatment of ZnO NPs, the polyphenol oxidase activity was significantly decreased. It should be highlighted that the amino acid, soluble protein and carbohydrates, and also Zn contents were highly enhanced in the callus treated with ZnO NPs (Abdel Wahab et al. 2020). Zn provided vital roles in different biochemical, physiological, and anatomical pathways. ZnONPs have been widely utilized in personal and medical care products, paints, coating materials, UV protectors, and absorber materials. However, these nano molecules might increase health and environmental risks because of their interaction with many biological and chemical biomaterials (Chithrani et al. 2006). Further research confirmed that the treatment of *Juniperus procera* cells with a suitable amount of biosynthesized ZnO NPs caused a significant enhancement in growth rate, chlorophyll A, and total protein contents (Salih et al. 2021). Interestingly, the treatment of callus of wheat and tobacco with ZnO NPs causes an increment in nutrient and protein contents respectively (Rizwan et al. 2019; Mazaheri-Tirani and Dayani 2020). It is further identified that ZnO NPs could modulate the expression of some genes encoded by certain proteins resulting in turn on/off the expression of some downstream genes (Salama et al. 2019). Also, zinc oxide NPs might increase the CAT activity in the callus of *Punica granatum* and *Prosopis glandulosa* (Farghaly et al. 2020; Hernandez-Viezcas et al. 2011). A strong correlation existed between CAT activity and Zn concentration might be revealed that the CAT enzyme is involved in defense response against ZnO-NPs or BP stress (Hernandez-Viezcas et al. 2011). Moreover, the strong correlations between LOX activity and Zn concentration were also confirmed in which ZnO–NPs could increase O_2^- formation causing oxidative stress (Manke et al. 2013). Upon the ZnO-NPs reaching into the mitochondria, they might induce ROS production by interfering with their reactions resulting in the depolarization of mitochondrial membranes (Xia et al. 2006). Of note, some enzymatic antioxidants were increased under ZnO NPs confirming these enzymes could be enabled plants to neutralize the stress. ZnO NPs provided some positive effects on the protein content of the callus of tomatoes even under salt stress (Alharby et al. 2016). Treatment of *Echinacea purpurea* callus extracts with biosynthesized ZnO NPs enhances secondary metabolite and anticancer activities (Karimi et al. 2018). In different concentrations, zinc as a micronutrient improves the efficiency of callogenesis and regeneration in Panicum virgatum (Shafique et al. 2020). ZnO NPs and ZnO submicron particles have been shown to improve onion *) Allium cepa L. 'Sochaczewska'(* seed germination and seedling growth in vitro. Seeds treated with 800 mgL1 of the NPs had the highest percentage of germination (Fig. 14.1a) (Tymoszuk and Wojnarowicz 2020). Zafar et al. (2016) reported Brassica nigra seed germination and seedling growth are affected with ZnO NPs concentrations ranging from 500 to 1500 mg/L, that also leads to improvement of antioxidative and non-antioxidants activities (Fig. 14.1b).

Silver (Ag)

Silver NPs are considered as one of the most important NPs produced worldwide and provide antimicrobial, cytotoxic, antifungal, physiological, and phytotoxic properties (Keller et al. 2013; Nel et al. 2006). Ag NPs are able to inhibit chronic contamination caused by microorganisms during plant culture experiments (Elechiguerra et al. 2005). These features came mainly from small size and unique phytochemical properties allowing Ag NPs to cross through biological membranes and organs and tissues to improve plant health (Kim et al. 2017). AgNPs significantly enhanced seed germination capacity and seedling growth rate in rice (*Oryza sativa* L., cv. Swarna) (Gupta et al. 2018). This NP has presented different applications in plant tissue technology including simultaneously improvement of callus induction, somatic embryogenesis, organogenesis, genetic transformation, somaclonal variations, and

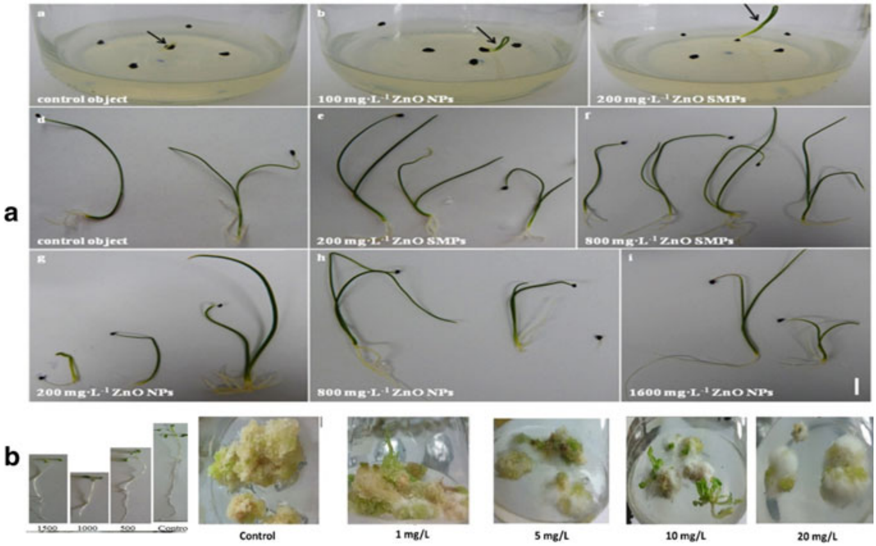

Fig. 14.1 Impact of ZnO NPs on seed germination and stem explants. *Allium cepa L. 'Sochaczewska'* (**a**), *Brassica nigra* (**b**). *Source* Tymoszuk and Wojnarowicz (2020), Zafar et al. (2016)

secondary metabolites production (Lateef et al. 2018; Adebomojo and AbdulRahaman 2020). In addition, AgNPs presented a high potential for improvement of growth, biomass, and secondary metabolites in plant cell cultures (Elechiguerra et al. 2005). It is identified that a suitable concentration of AgNPs can significantly induce the callus formation, the regeneration of shoot and roots, and the nursery phase during the propagation of banana (Musa ssp.) (Huong et al. 2021) of note Ag-SiO_2 stimulates the production of artemisinin in the roots of *Artemisia annua* (Zhang et al. 2013). Moreover, biologically synthesized AgNPs can increase the callus fresh weight and also callus formation in the leaf explants of *Solanum nigrum* (Ewais et al. 2015). Another recent report identified that supplementation of AgNPs and plant growth regulators sustainably enhanced the callus proliferation, biomass, antioxidant, and secondary metabolites production during in vitro culture of *Caralluma tuberculate*. While the sole application of AgNPs produced a higher amount of antioxidants and secondary metabolites (Ali et al. 2019b). On Nicotiana tabacum, hormone-stabilized AgNPs fully promoted the roots (a) control water treatment, (b) IAA, (c) IBA, (d) AgIAA, (e) AgIBA) (Fig. 14.2a). (Thangavelu et al. 2018) Silver NPs in concentrations ranging from 1 to 5 ppm were found to be effective on banana (*Musa spp.*). In vitro shoot cultures on media containing 3 ppm AgNPs also produced a significant number of roots (Fig. 14.2b) (Do et al. 2018).

Gold (Au)

The incorporation of Au NPs into the callus medium of *Arabidopsis thaliana* could improve the seed germination, seedling growth capacity, pod length, and a number of

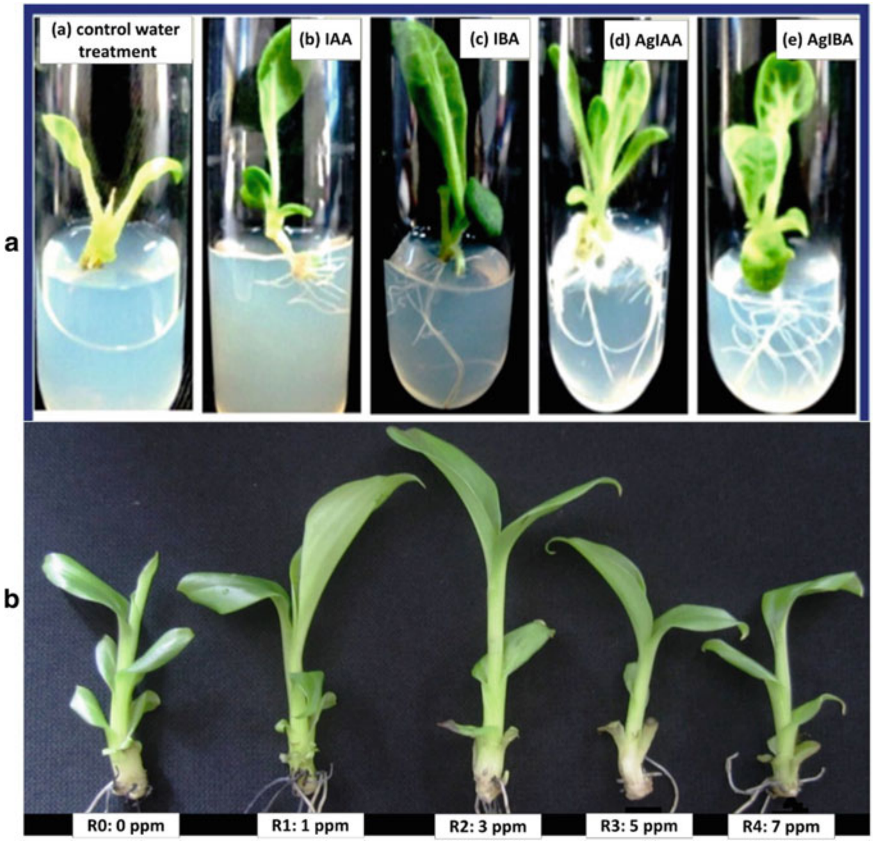

Fig. 14.2 Effect of AgNPs on rooting. *Nicotiana tabacum* (**a**), on banana (Musa spp.) (**b**). *Source* Thangavelu et al. (2018), Do et al. (2018)

seeds. Moreover, the use of Au NPs might enhance the antioxidant enzyme activity in the *A. thaliana* through the decrease of microRNA expression levels miR398 and miR408) (Kumar et al. 2013). Further reports confirmed that the treatment of cell suspension cultures with Au NPs increases the intracellular free amino acid pools (alanine, valine, and γ-aminobutyric acid) and also modulates the extracellular proteins composition (Selivanov et al. 2017).

Copper (Cu)

The treatment of callus culture of *Mentha longifolia* with Cu and Co NPs has a positive impact on the improvement of fatty acid contents in which the linalool and linalyl acetate contents were higher in the treated cells (Talankova-Sereda et al. 2016). Like the other NPs, the use of CuO NPs in the *O. basilicum* callus cultures could elicit the biosynthesis of bioactive compounds with a high antioxidative capacity.

Moreover, the accumulation of flavonoid and phenolic molecules was also significantly improved in the media supplemented with CuO NPs. In addition, the SOD and POD (Peroxidase) activities were highly elicited in the CuO NPs treated cultures compared to the control. Notably, the HPLC data identified that the production of rosmarinic acid, chicoric acid, and eugenol was improved when the callus cultures of *O.basilicum* were treated with CuO NPs (Nazir et al. 2021). (Paramo et al. 2020) suggested that the positive impact of Cu NPs is due to copper showing a greater positive effect in the physio-biochemical processes such as hormone signaling pathways, metabolism, and electron transport reactions. However, the increase in NPs concentrations might show some negative effects on biomass production. Another report showed that CuO NPs could stimulate the in vitro induction of bioactive compounds in the suspension cells of *Stevia rebaudiana* (Javed et al. 2017b). While the use of five levels of CuO NPs caused a significant decrease in fresh and dry weight, water content, amino acids, and potassium contents in the callus cells of *Solanum nigrum* (Abdel-Wahab et al. 2019). Capped CuO NPs were more toxic for the callus cells of *Trigonella foenum-graecum* than uncapped forms causing a higher production of secondary metabolites (ul Ain et al. 2018). It should be noted that CuO NPs could be elicited biomass and bioactive compounds accumulation, and antioxidants biosynthesis in callus cultures of *Ocimum basilicum* (Nazir et al. 2021).

Carbon Nanomaterials (CNMs)

Today, carbon nanomaterials (CNMs) have been attracted much more attention for their application in plant biology. These materials have exhibited positive potential for regulating the plant growth capacity which was a promising future for agriculture. However, the precise mechanism of CNMs in plants is yet well understood especially at the molecular levels (Zhenjie et al. 2020). Until now, the potential different CNMs such as carbon nanotubes and graphene have been evaluated in plant biology research. The appropriate concentration (25–500 μg mL^{-1}) of multi-walled carbon nanotubes can highly improve the callus growth rate in the leaf explants of *Satureja khuzestanica*. Whilst, the higher amounts (100–500 μg mL^{-1}) of these nanotubes might decrease the callus biomass production (Ghorbanpour and Hadian 2015). Similarly, the incorporation of about 100 μg/mL of multi-walled carbon-related nanotubes significantly increased the callus growth cates in the tobacco explants. It is believed these activities are achieved through the upregulation of the genes involved in cell division and extension, cell division, and water transport (Khodakovskaya et al. 2012). Of note, multi-walled carbon nanotubes could intensely improve the nitrogenase activity and also increase gene expression levels involved in the regulation of nodules development (Yuan et al. 2017). However, the treatment of *Arabidopsis* cell cultures with 10–600 mg/L of carbon nanotube treatment was decreased the viability and dry weight of plant cells (Lin et al. 2009). The exposure to low concentrations of single-walled carbon-related nanotubes provided drought stress induced by polyethylene glycol through the activation of some antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), and Ascorbate peroxidase (APX) and also biosynthesis of secondary metabolites (ie., phenols and proline) in the seedlings of *Hyoscyamus niger* (Hatami et al. 2017). Graphene-related nanomaterials provided some impressive characteristics such as two-dimensional structure, unique electronic and optical attributes, mechanical flexibility, electrical conductivity, and high and chemical stability resulting in greatly broadened applications in biology, chemistry, and medicine (Shehzad et al. 2016; Shen et al. 2016; Dreyer et al. 2010).

Iron (Fe)

It is reported that the use of FeO NPs in the medium of *Hyoscyamus reticulatus* could increase the production of tropane alkaloid hairy roots through the induction of oxidative stress reactions (Moharrami et al. 2017). Further research identified that SiO2 and Fe NPs could significantly enhance the accumulation of some essential pharmaceutical biologics including rosmarinic acid and xanthomicrol in the hairy roots of *Dracocephalum kotschyi* (Nourozi et al. 2019a, b).

Silicon (Si)

Silicon (Si) as the second most frequent element is enabled of protecting plants from biotic and abiotic stresses, decreasing transpiration losses, and improving their resistance to different diseases (Liang et al. 2007; Ma 2004; Nawrot et al. 2010). It is identified that the treatment of rice cell cultures with silica NPs noticeably reduced Cd toxicity by a decrease in silica NPs size. Moreover, silica NPs could respectively increase and decrease the Si and Cd uptake capacities allowing the alleviation of Cd toxicity in the cells (Cui et al. 2017). It is reported that the fluorescein isothiocyanate-labeled mesoporous silica NPs (MSNs) could successfully interact with hybrid suspension cells of *Liriodendron* through the internalization of MSNs via endocytosis. Owing to admirable biocompatibility, MSNs might be considered as a potential nanocarrier for walled-plant cells (Xia et al. 2013).

Ca

CaO NPs are vital elements that functioned as transducers in several adaptive and developmental reactions in plants. These elements could enhance the tolerance of callus of *Triticale* against salt stress through the improvement of biochemical activity (Yazıcılar et al. 2021).

SnO_2

The cytotoxic effects of SnO2 and Ag/SnO2 NPs on the *tobacco* cell cultures identified the importance of structural modifications on the toxic properties of NPs. Indeed, SnO2 NPs presented low toxicity while Ag-doped NPs have a significant effect in inhibiting the toxicity through modulation of oxidative stress pathways in tobacco cells. Microscopic analyses demonstrated a high level of cell mortality upon treatment with a high level of SnO_2 NPs (e.g., 0.5 mg/ mL) or even a low concentration of Ag/SnO2 NPs (e.g., 0.2 mg /mL). Further experiments showed these components could significantly enhance the accumulation of neutral red stain into the vacuole of NPs-treated tobacco cells inducing the high acidification (Mahjouri et al. 2020).

Polymeric Nanoparticles

Polymeric NPs are colloidal nano molecules ranging from 1 to 1000 nm which are generally prepared from biodegradable polymers (Prabha et al. 2020; Bhattacharjee et al. 2016). Biodegradable polymers mainly utilized for the polymeric NPs fabrications such as poly (lactide) (PLA), poly (amino acids), poly (lactide-co-glycolide), poly (ε-caprolactone) (PCL), (PLGA) copolymers, and several natural polymers especially alginate and chitosan (Asti and Gioglio 2014). Notably, polymeric NPs presented some important advantages such as biocompatibility, biodegradability, simple and easy fabrication process, non-toxicity, non-immunogenicity, and capability to site-specific targeting organs or tissues (Jawahar and Meyyanathan 2012). Recently, polymeric NPs have extensively been implicated in the production of pesticides, herbicides, fertilizer, and plant growth regulators. It is reported that 2,4-D loaded PLGA NPs could significantly increase the growth rate and biomass of Medicago sativa cell suspension cultures compared to its free form (Poyraz et al. 2021). Furthermore, the potential of bulk or nano-chitosan components, as an eco-friendly natural nano-molecule, has been evaluated in morphogenesis, growth, micropropagation, and physiology of Capsicum annuum suspension cells. The treatment of suspension cells with bulk chitosan or synthesized chitosan/tripolyphosphate (TPP) NPs were manipulated morphology and differentiation of some tissues and organs, especially the root architecture. Of note, the appropriate concentration of nano-chitosan might trigger organogenesis through micropropagation (Asgari-Targhi et al. 2018). The chitosan NPs synthesized by *Penaeus semisulcatus* shrimp shells could strongly inhibit some bacterial and fungal pathogens. In addition, chitosan NPs may use to develop pesticides against mosquito vectors in food packaging applications (Thamilarasan et al. 2018).

Dendrimer Nanoparticles

Cationic polyamidoamine (PAMAM) dendrimers as a highly branched NP could be utilized for the improvement of gene delivery capacity into the different cells. In fact, PAMAM may interact with DNA molecules allowing protection from ultrasonic damage. The use of PAMAM could intensely improve the transformation and gene expression efficacy in the *alfalfa* cells (Amani et al. 2018).

Quantum Dots (QDs)

QDs are fluoresce-based NPs expressed with bright and pure colors upon excitation with UV wavelength (Whiteside et al. 2019). The treatment of the suspension culture of *Medicago sativa* with mercaptopropionic acid-coated CdSe/ZnS QDs to the suspension culture significantly reduced cell growth rate. Subsequently, a high accumulation of the CdSe/ZnS QDs in the cytoplasm and nucleus led to dose- and time-dependent production of ROS (Santos et al. 2010). Further data showed these cytotoxic and genotoxic features were induced by the activation of DNA-related repair genes and ROS-eliminating enzymes (Santos et al. 2013). The below table shows the effect of some NPs on plant cell and tissue culture (Table 14.1).

Table 14.1 The effect of some NPs on plant cell and tissue culture

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Ag	Seedling	Rice *(Oryza sativa* L., cv. Swarna*)*		10, 20, 40 ppm	Carotenoid and chlorophyll contents improved	Gupta et al. (2018)
Ag-SiO_2	Root	*artemisinin*	101.8 ± 8.9	900 mg/L	Increased lipid peroxidation and MDA and CAT accumulation	Zhang et al. (2013)
Ag	Callus	*Valeriana officinalis* L	35	25, 50, and 100 μg/mL	Inhibited bacterial infections	Abdi et al. (2008)
Ag	Callus	*Caralluma tuberculata*	40	30, 60 and 90 μg/l	Enhanced callusproliferation and biomass	Ali et al. (2019b)
Ag	Shoot	*Tecomella undulata (Roxb)*		5 to 80 mg/L	Improved the number of fresh shootsper explants	Aghdaei et al. (2012b)
Ag	Callus	*Calendula officinalis*		0.3 mg/L	Increased essential oils production capacity	Abbasi Khalaki et al. (2016)
Ag	Cell suspension	*Echinacea purpurea*		0, 2 and 4 mg/L	enhanced cichoric acid production	Ramezannezhad et al. (2019)
Ag	Cell suspension	*Linum usitatissimum*	18	1, 5, 10, 20, 30, 40, 50 μg/l,	Improved lignans and neo-lignansactivities	Zahir et al. (2019)
Ag	Callus	*banana (Musa ssp)*		0.0, 2.0, 4.0 and 6.0 ppm	Improved callusformation, regeneration, and multiplication	Huong et al. (2021)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Ag	Cell suspension	*Corylus avellana* L		0, 2.5, 5, and 10 ppm	Decreased the viability of the cells and increased radical scavenging activities	Jamshidi et al. (2016)
Ag	Shoot culture	*Vanilla planifolia*	15–35	0, 25, 50, 100 and 200 mg/L	TPC and antioxidant enzymesactivities increased	Spinoso-Castillo et al. (2017)
Ag	Hairy root	*Brassica rapa*			Phenolic compounds enhanced	Chung et al. (2018b)
Ag ion (Ag+)	Hairy root	*Cucumis anguria*		2.0 mg/L	Induced hairy rootand phenolic formation	Chung et al. (2018a)
Ag	Hairy root	*Datura metel*	50–60	20 mg/L	Enhanced atropine contents	Shakeran et al. (2015)
Ag	Cell suspension	*Capsicum frutescens*		3.0 mg/L	Capsaicin production enhanced	Bhat and Bhat (2016)
Ag + Au	callus	*Prunella vulgaris* L		different ratios (1:2, 1:3, 2:1, and 3:1)	Improved TPC and TFC contents	Fazal et al. (2016)
Ag + Au	Cell suspension	*Lavandula angustifolia*	Au = (24) nm and Ag = (27) nm	10–50 mg/dm	Decreased lower molecular weight compounds	Wesołowska et al. (2019)
Ag + plant growthregulators (PGRs)	Callus	*Caralluma tuberculata*	40	30, 60 and 90 μg/l	Improved proliferationand callusbiomass	Ali et al. (2019b)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Au and Ag	Callus	*Linum usitatissimum* L		500 mg /L	Increased somaclonal variationscallus	Kokina et al. (2017b)
Au	*Seedling*	*Arabidopsis thaliana*	24 nm	10 and 80 µg/ml	Enhancedseed germination, vegetative growth, and free radical scavenging capacity	Kumar et al. (2013)
biosynthesized nanosilver (BNS)	Callus	*Ocimum*	12–80 nm	10, 50 and 100 mg/L	Used for surface sterilizationof explantand callus	Adebomojo and AbdulRahaman (2020)
CaO + on exposed to short and long-term salt stress	Callus	*Triticale*		1.5 ppm Ca2 + NPs concentration of 50 g and 100 g NaCl	Suppressed the side effects of NaCl stress	Yazıcılar et al. (2021)
Carbon nanotube	Suspension	*Arabidopsis*		10–600 mg/ L	Cell viability rate and dry weight decreased	Lin et al. (2009)
Co	Cell suspension	*Artemisia annua*	10 nm	0.25, 2.5, and 5 mg/L	Reduced expression levels of SQS and DBR2 genes	Ghasemi et al. (2015)
Cu and Co		*Mentha longifolia*		Copper (0.5 mg/L) and cobalt (0.8 mg/L)	Improved microplant height and growth rate	Talankova-Sereda et al. (2016)
CuO + CaO + zno + Under In Vitro Salt Stress	Callus	*Alfalfa (Medicago Sativa L.)*	CaO = 35 to 160 nm CuO = 20–45 nm ZnO = 17–65 nm	50 mM treatment NaCl with 0.8 ppm NPs	Protectedthe callusagainst NaCl stress	Simsek et al. (2021)
Cu-Au bimetallic	Adventitious root culture	*Stevia rebaudiana*			TPC and TFC increased	Ghazal et al. (2018)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Cu-NPs + copper sulfate		*Ocimum basilicum*	20–40 nm	0.1, 2.5, 5, 7.5, 10, 12.5 and 15 μM	Elevate regenerationcapacity	Ibrahim et al. (2019)
CuO	Cell suspension	*Brassica rapa ssp.*	25–55 nm	50, 250, and 500 mg/L	Reduced total chlorophyll, carotenoid, and sugar contents and improved proline and anthocyanins	Chung et al. (2019)
CuO	Callus	*Solanum nigrum L*	*> 50 nm*	*50, 100 and 150 mg/L*	Decreased fresh weight, amino acid and potassium contents	Abdel-Wahab et al. (2019)
CuO	Shoot and root	*Withania somnifera*		1 ppm	TPC, TFC, and tannins contents improved	Genady, Qaid et al. (2016)
CuO + MnO	Callus	*Ocimum basilicum (Thai basil)*	CuO-NPs (20–50 nm) + MnO-NPs (20–30 nm)	(1, 5, 10, 25, 50, 100 mg/L)	Increased phytochemicals accumulation	Nazir et al. (2021)
$CuSO_4$	Callus	*Verbena bipinnati da Nutt*		5–15 mg/L	Shoot and root lengths, phenolic contents, and fresh weight increased	Genady et al. (2016)
$Fe2O_3$	Hairy root	*Dracocephalum kotschyi Boiss*		75 mg/L	Flavonoid contents increased	Nourozi et al. (2019b)
Fe	Hairy root	*Dracocephalum kotschyi*	100		Flavonoid contents increased	Nourozi et al. (2019a)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Fe_3O_4 ZnO	Hairy root	*Cichorium intybus*		50 mg/L	TPC and TFC improved	Mohebodini et al. (2017)
Fe_3O_4	Hairy root	*Hyoscyamus reticulatus* L		450 and 900 mg/L	Improved scopolamine and hyoscyamine	Moharrami et al. (2017)
Fe3O_4	Cell suspension	*Hypericum perforatum* L	0, 50, 100 and 150 ppb		Hyperforin and hypericin enhanced	Sharafi et al. (2013)
Mgo + thidiazuron *(TDZ)*	Seed	*Raphanus sativus* L		20 mg/L	Biomass, TPC, TFC and antioxidant activity increased	Hussain et al. (2019)
Mn_2O_3	Shoot-tip	*Atropa belladonna*		25 mg/L	Increased production of alkaloids and flavonoids	Tian et al. (2018)
MWCNTs	Callus	*Satureja khuzestanica*		0, 25, 50, 100, 250 and 500 µg/ ml	Reduced the callus biomass	Ghorbanpour and Hadian (2015)
MWCNTs	Seedlings	*Salvia verticillata* L		(0–1000 mg /L	Flavonoid contents increased	Rahmani et al. (2020)
nano-garphene oxide(NGO) + under polyethylene glycol-induced dehydration	Callus	*Plantago major* L		100–800 µg/ mL	Reduced growth rate and osmotic potential capacity	Ghorbanpour et al. (2018)
Perlite NPs TiO_2/perlite nanocomposites	Callus	*Hypericum perforatum*	14.51–23.34 and 15.50–24.61 nm	25–200 mg/L	Enhanced the variety, quantity and number of volatile compounds	Ebadollahi et al. (2019)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Poly(amidoamine) dendrimer	Callus	*Agrostis stolonifera* L	4.5 nm		Improved gene transformation efficacy	Pasupathy et al. (2008)
Silver NPs and silver salt ($AgNO_3$)	Callus	Two varieties of wheat (*Triticum aestivum* L.)	17 ± 5 nm	0, 20, 40, 60 ppm	Improved the growth rate, callus formation, protein contents, and gene expression levels	Barbasz et al. (2016)
AgNP	Leaves	Chrysanthemum		250 ppm	Teatment for 15–20 min proved optimal for controlling the contamination	Tung et al. (2021)
SiNP	Cell suspension	Rice	19 nm, 48 nm and 202 nm		decreased cadmium toxicity	Cui et al. (2017)
SMF Fe_2O_3	Cell suspension	*Dracocephalum polychaetum Bornm*	30 mT 100 ppm		Flavonoids, lignin, anthocyanins and malondialdehyde production increased	Taghizadeh et al. (2019)
TiO_2	Embryonic callus	*Cicer arietinum*		0/5.1.5,3,4.5.6 mg/L	Total phenolic compounds increased	Al-Oubaidi and Kasid (2015)
TiO_2 and ZnO	Cell suspension	*Linum usitatissimum*	30 10–30	0–150 mg/L 0–120 mg/L	The activity of PAL and CAT enzymes enhanced	Karimzadeh et al. (2019)
TiO_2/perlite	callus	*Hypericum perforatum*	300–350	25–200 mg/L	Increased volatile compounds	Ebadollahi et al. (2019)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Tio_2, NH_4NO_3, Ag	Cell suspension	*Aloe vera*			Secondary metabolitesproduction improved	Raei et al. (2014)
Zinc and iron nano-oxides	Cell suspension	*Hypericum perforatum*		0, 50, 100 and 150 ppb	Enhanced hyperforin production	Sharafi et al. (2013)
Zinc oxide nano and bulk particles	Callus	*Punica granatum*	100 nm and surface area about 15–25 m^2g-1	0, 10, and 150 µg/mL	Improved CAT, SOD, POD, APX, and PPO activities	Farghaly et al. (2020)
ZnO	Callus	*Panicum virgatum*	90–390	10–50 mg/L	Improvedthe regenerationcapacity and callogenesis	Shafique et al. (2020)
ZnO	Callus	*Solanum nigrum calli*		0, 50 and 100 mg/L used)	The activities of lipoxygenaseand antioxidant enzymesimproved	Abdel Wahab et al. (2020)
ZnO	Callus	*Brassica nigra*	100	1–20 mg/L	Enhanced secondary metabolitecontents in callus and seedlings	Zafar et al. (2016)
ZnO	Callus	*Echinacea purpurea*		75 mg/L	Production of anticancer components and flavonoid contents improved	Karimi et al. (2018)
ZnO	Seedling	*Brassica nigra*		500–1500 mg/L	Prohibitedseed germination	

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
ZnO	Cell suspension	*Linum usitatissimum*	35	1, 5, 10, 20, 30, 40, 50, 75, 100, 200 and 500 µg/l	Increased lignans and neolignans components	Abbasi et al. (2019)
ZnO	Seedling	*Chenopodium quinoa*		0.2, 2, 10, 20 mg/L	Germinationrate, shooting capacity improved	Al Gethami and El Sayed (2020)
ZnO	callus	*Thymus Kotschyanus* *Tymus daenensis,* *Thymus vulgaris*		100 and 150 mg/L	Enhanced thymol and carvacrol contents	Mosavat et al. (2019)
ZnO	Shoot culture	*Stevia rebaudiana*	34	0, 0.1, 1.0, 10, 100 or 1000 mg/L	steviol glycosides, avonoid and phenolic contents improved	Javed et al. (2017b)
ZnO	Shoot	*Lilium ledebourii*		20–100 mg/L	Anthocyanin, phenolics and flavonoids increased	Chamani et al. (2015)
ZnO	Hairy root	*Hyoscyamus reticulatus*		100 mg/L	Enhanced total phenolic contents and alkaloids	Asl et al. (2019)
ZnO CuO	Root culture	*Stevia rebaudiana*		20–30 25–30	Steviol glycosides and flavonoids enhanced	Ahmad et al. (2020)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
ZnO and CuO	Callus	*Stevia rebaudiana*		1–10 mg/L	Increased total phenolic, flavonoid, antioxidant contents, and DPPH scavenging capacity	Javed et al. (2018)
Zno using Phoenix dactylifera)	Callus	*Juniperus procera*	17 to 36	0.0 mg/L, 80 mg/L and 160 mg/L	Improved callusformation	Salih et al. (2021)
ZnO, SiO_2, and Fe_3O_4	Callus	*Salvadora persica*	25 to 55	of 0.5 or 2 mg/L	Increasing biomass	Fouda et al. (2021)

14.3 Mechanism of Improvement of Secondary Metabolism by Nanoparticles

Elicited plant cell and suspension cultures have attracted more attention worldwide because of their capacity for the production of industrially vital secondary metabolites (Ali et al. 2019a). Plant secondary metabolites are organic components which involved directly in the growth, development, and reproduction of plant cells and tissues. Moreover, these molecules are contributed to different signaling cascades, and also defense pathways against several microorganisms, pathogens, and insects (Hartmann 2007). Most of the secondary metabolites are considered as an enriched resource of pharmaceutical molecules with defensive properties in the human body (Zhao et al. 2005a, b). The biosynthesis of secondary metabolites is dependent on biotic and abiotic factors such as growth rate, physiology, light intensity, temperature, and humidity. Moreover, the secondary metabolite productivity of callus cultures has been especially dependent on culture media composition, pH, agitation, aeration, and light density (Ochoa-Villarreal et al. 2016; Isah et al. 2018). Nowadays, several various biotic and abiotic factors have been evaluated to induce the production and concentration of the secondary metabolites and also increment cell volume in plant suspension cultures (Rao and Ravishankar 2002). Many NPs could be activated through enzymatic pathways which are responsible for secondary metabolites production (Wang et al. 2021). Nanomaterials could be considered a novel effective abiotic for the stimulation of biosynthesis of secondary metabolites (Fakruddin et al. 2012). Different reports have been identified that the nanomaterials could increase the expression of several genes involved in the biosynthesis of secondary metabolites (Ghasemi et al. 2015; Yarizade and Hosseini 2015). Titanium oxide NPs, for example, could distinctly increase the production of gallic acid, cinnamic acid, chlorogenic acid, tannic acid, and o-coumaric acid in the embryonic callus of *Cicer arietinum* (Mohammed 2015). Moreover, the use of silver NPs might increase the concentration of artemisinin in the hairy root cultures of *Artemisia annua* (Zhang et al. 2013). Notably, the growth rate of calli of *Satureja khuzestanica* was significantly improved when treated by gradually increasing concentrations of carbon nanotubes in the plant medium (Ghorbanpour and Hadian 2015). While, in the higher concentration of carbon nanotubes (i.e., 500 mg/L), the highest amounts of H_2O_2, PPO, POD, and secondary metabolic activities were observed. Similarly, the use of about 250 and 1000 mg/L CeO_2 and also indium oxide NPs caused excessive ROS production and PAL, and PPO in the *A. thaliana* suspension cells which revealed the possible function of secondary metabolites against oxidative stresses (Ma et al. 2016). Although NPs could implicate positive impacts on some signaling pathways and modulate the metabolism of secondary compounds, the precise mechanisms of these reactions were not understood. It is believed that the initial responses of different plants to the NPs might be elevated levels of ROS, cytoplasmic calcium and subsequent upregulation of mitogen-activated protein kinase (MAPK) cascades observed during abiotic stresses (Sosan et al. 2016). The increase of Ca^{2+} levels is associated with upregulating some protein signaling pathways in the *O. sativa* roots treated with AgNPs

(Mirzajani et al. 2014). It is hypothesized that AgNPs might impede cell metabolism through binding to the Ca^{2+} receptors, Ca^{2+} channels, and Ca^{2+}/Na^{+} ATPases. Moreover, NPs could minimize Ca^{2+} or signaling molecules in the cytosol upon sensing calcium ions by calcium-binding proteins or other NP-specific proteins (Khan et al. 2017). Further data identified that MAPK phosphorylation and also the activation of downstream transcription factors led to induce of transcriptional reprogramming of secondary metabolism in many plants (Vasconsuelo and Boland 2007; Schluttenhofer and Yuan 2015; Phukan et al. 2016). Although the exact evidence for the contribution of MAPK pathways in plant-NP interactions is yet identified, analogous pathways involved in AgNP-induced signaling reactions were found in the animal and human cell line studies (Eom and Choi 2010; Lim et al. 2012). In this sense, it is believed that plants might utilize MAPK pathways upon exposure to the Ag NPs (Kohan-Baghkheirati and Geisler-Lee 2015). Recent data confirmed that NPs could be regarded as a nutrient resource or an elicitor inducing the overproduction of secondary metabolites (Kim et al. 2017). For instance, the treatment of the tobacco cell suspension cultures with different concentrations of Al_2O_3 NPs could accumulate phenolic molecules (Poborilova et al. 2013). Similarly, the addition of Ag-SiO_2 core–shell NPs into the *Artemisia annua* hairy root cultures could intensely improve artemisinin content (Zhang et al. 2013). Multi-walled carbon nanotubes could significantly induce the production of total phenolics, flavonoids, rosmarinic acid, and caffeic acid in the *Satureja khuzestanica* callus cultures compared to the control experiments (Ghorbanpour and Hadian 2015). The cultures supplemented with zinc nano-oxide showed an increased amount of hypericin and hyperforin (Sharafi et al. 2013). It should be noted that recent genomic data have been found that plants might respond to the internalization of nanomaterials similar to the biotic or abiotic stresses (Khodakovskaya et al. 2012; Kohan-Baghkheirati and Geisler-Lee 2015). Indeed, NPs could be modulated the secondary metabolites production through the induce of several signal transduction pathways including calcium flux, overproduction of ROS, and MAPK phosphorylation reactions (Mahjouri et al. 2018). It seems that NP-induced ROS can function as a signal to trigger the plant's secondary metabolism (Marslin et al. 2017). Plants could produce different types of ROS such as H_2O_2, superoxide, hydroxyl radical, and singlet oxygen during the detoxification mechanism. Different antioxidant enzymes (oxidoreductases and CAT), hormones (e.g., abscisic acid and salicylic acid), and antioxidants with low molecular weight (thiols and ascorbate) are involved in the neutralization of these toxic molecules. Notably, excessive ROS might lead to increase lipid peroxidation capacity, electrolyte leakage, and finally, DNA degradation caused cell death (Dev et al. 2018; Tripathi et al. 2017). It is believed that callus, cell suspension, and hairy root cultures could be considered as an advanced strategy for the production of therapeutically important plant alkaloids (Moreno et al. 1995; Goldhaber-Pasillas et al. 2014). For example, the hairy root cultures of *Catharanthus roseus* caused the significant production of indole alkaloids such as horhammericine, catharanthine, lochnericine, and tabersonine (Li et al. 2011). Moreover, different alkaloids such as ajmalicine, serpentine, antirhine, cathindine, acuamicine, and lochnericine have been successfully obtained from the plant calli, cell suspensions, sprouts, pilose roots, somatic embryos, and vincristine in sprouts and embryos (van Der Heijden et al. 2004; El-Sayed and Verpoorte 2007; Almagro et al. 2014). In fact, the activation of signaling pathways could modulate the gene expression levels which followed by continuous enzymatic reactions resulting in consecutively change in secondary metabolites production. Previously reported that any change in the activity of phenylalanine ammonia lyase, polyphenol oxidase, and peroxides could modulate the biosynthesis of secondary metabolites (Hatami et al. 2016). The influence of NPs on biosynthesis of secondary metabolites in plant cell and tissue cultures is shown in Fig. 14.3.

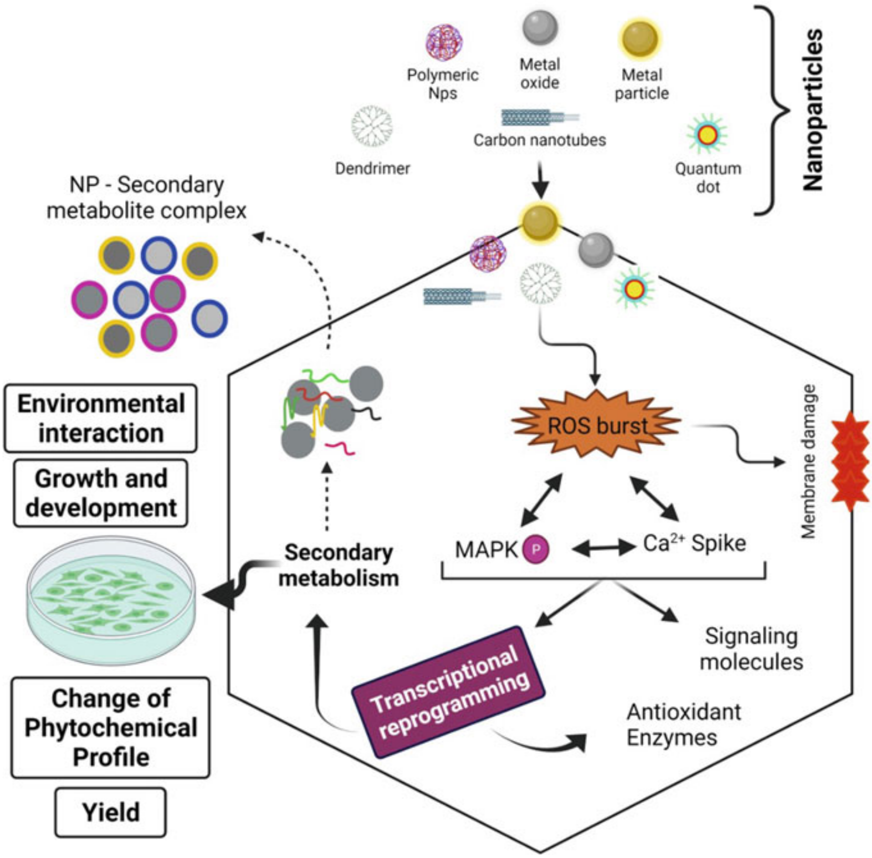

Fig. 14.3 The effect of nanoparticles on the synthesis of secondary metabolites in plant cell and tissue cultures

14.4 Mechanisms of Nanoparticles Affecting Callus

The callus culture could provide the required sterilized and reliable large-scale resources of plant materials for the synthesis of NPs have positive impacts on the callus physiology and secondary metabolites pathways through the production of oxidative stress which eventually activated plant metabolic reactions to inhibit the oxidative outbursts through the production of phytochemicals (Choi and Hu 2008). Biotic and abiotic stresses might suppress cell differentiation during callogenesis through the unwanted production of ROS or the production of toxic metabolites injured directly by the plant cells (Srinivasan 2007). Plant cells could fight against oxidative stress through several enzymes such as SOD, CAT, POD, and APX in which they scavenged the free radicals during cell division (Abbasi et al. 2011). It should be noted that the inclusion of different NPs into the tissue culture media might improve the morphogenetic potential of treated explants (Mandeh et al. 2012). The optimum concentration of AgNPs could improve the callus induction and biomass in the explants of *Phaseolus vulgaris* (Mustafa et al. 2017). While the precise physiological and molecular responses of this impact are yet understood, it is speculated that AgNPs may enhance the nutrient and water uptake capacity from the culture media by mutilating the plant cell wall (Ali et al. 2018). The chemical composition of NPs is mainly responsible for the motivation or inhibition effects of metallic oxide NPs on the callus cells and also the stresses induced by the size, shape, and surface of these NPs. It should be highlighted that the mechanism of transferring NPs across the cell membrane is not well understood, but it is believed that the use of NPs could increase the lipid membrane peroxidation induced by enhancement of ROS production and upregulation of MAPK cascades (Marslin et al. 2017). Moreover, size reduction, surface area enhancement, and capability of apoplastic or symplastic transportation could lead to more electrostatic interactions of many NPs with the living cell membranes resulting in the activation pathways for the biosynthesis of secondary metabolites in the plant cells (Javed et al. 2017a). Upon exposure to NPs, plant cells suffered a series of cascade reactions resulting in oxidative outbursts, ROS generation damage, and subsequent disruption of cell membrane and nuclei. Plants have activated their metabolic pathways such as secondary metabolites induction and MAPK cascades to inhibit intense stress situations and improve the ROS scavenging capacity (Sinha et al. 2011). CAT and APX antioxidant enzymes could significantly scavenge ROS and play a crucial role in the mitigation of oxidative stress (Garg and Manchanda 2009). It should be highlighted that the precise physiological and molecular responses of plant suspension and callus cells to the NPs are still unclear (Bezirğanoğlu 2017; Elmaghrabi and Ochatt 2006).

14.4.1 Impact of Nanoparticles on Quantitative and Qualitative Features of Calli

The treatment of *Salvadora persica* callii with ZnO, SiO2, and Fe3O4NPs increased callus growth rates and improved the production of constituent benzyl isothiocyanate. Further data identified that the increment of benzyl isothiocyanate activity was associated with the decrease of H_2O_2 content and the increase in the activity of superoxide dismutase and peroxidase. Moreover, the genomic DNA stability was reduced when higher doses of NPs utilized (Fouda et al. 2021). CuO, ZnO, and CaO NPs could present an effective approach for the protection of alfalfa callus against NaCl stress (Simsek et al. 2021). The treatment of wheat and *Stevia rebaudiana Bertoni* calli with ZnO NPS could increase proline concentration, flavonoid contents, and antioxidant enzyme (Javed et al. 2018; Barbasz et al. 2016). Exposure of Zn and ZnO NPs on callus cultures of bananas induced a significant decrease in growth rate but it enhanced the total proline associated with CAT, SOD, and POD activities. Despite the antifungal and antibacterial properties, further analyses confirmed NPs have no negative effects on explants regeneration (Helaly et al. 2014; Rad et al. 2020). Ag NPs could present positive effects on plant organogenesis through the inhibition of ethylene production. Upon exposure to Ag NPs, the number of shoots, their lengths, and the percentage of produced shoots were substantially enhanced in the nodal explants of *Tabernaemontana undulata* (Aghdaei et al. 2012c).

14.5 Some Important Applications of Nanomaterials in PTC

14.5.1 Somaclonal Variation

Generally, any changes in chromosome structure and number, DNA sequence, DNA arrangement, and transposable elements activation have been known as somaclonal variation (Kim et al. 2017). Moreover, somaclonal variation is proposed for the description of the plant tissue culture-induced phenotypic and genotypic variation in regenerated plants (Ngezahayo et al. 2007). Indeed, this parameter could evaluate the genetic and epigenetic variation that existed between clonal regeneration and the relative plant (Kaeppler et al. 2000; Wang and Wang 2012). It is identified that the use of gold and silver NPs could in vitro evaluate somaclonal variation in the coding sequence of methylesterase and also Mlo-like protein during tissue developmental stages of donor plant, calli, and regeneration in the *Linum usitatissimum* (Kokina et al. 2017b). Moreover, the treatment of *Vanilla planifolia* plantlets with different concentrations of AgNPs induced changes in repeat units and also polymorphism in its nuclear genome. Interestingly, the polymorphism percentage was enhanced by the increase in the concentration of AgNPs (Bello-Bello et al. 2018). Of note, the addition of AgNPs to the culture medium induced variation in morphology, anatomy, protein content, and DNA profile of *Solanum nigrum* calli (Ewais et al. 2015). Somaclonal variations might create plants associated with several key features such as higher secondary metabolite production and more resistance to stresses (Kim et al. 2017).

14.5.2 Organogenesis

Different NPs (Au and Ag) could be effective on the inhibition or induction of regeneration capacity and growth of adventitious organs such as roots and shoots through the inhibition of ethylene production (Kim et al. 2017). It is confirmed that tobacco root cells could directly uptake AgNPs resulted in significant adventitious roots formation (Cvjetko et al. 2018). Moreover, the treatment of *S. viarum* and *Gentiana lutea* cells with silver nitrate NPs might induce root formation (Purine et al. 2015; Petrova et al. 2011). Further reports showed that suitable concentrations of AgNPs and AuNPs have positive effects on the random organogenesis in chrysanthemums, gerbera, and cape primrose (Tymoszuk and Miler 2019). It should be noted that shoot induction percentage and also their lengths were significantly improved upon the treatment of stem and nodal explants of *Tecomella undulata* treated with AgNPs (Aghdaei et al. 2012a).

14.5.3 Somatic Embryogenesis

Somatic embryogenesis, developed from somatic cells, is an effective method for micropropagation, regeneration of new plants, and genetic improvement of plant cells (Aghdaei et al. 2012a). Figure 14.4 Cu-NPs could significantly trigger the regeneration capacity of Ocimum basilicum through somatic embryogenesis. Indeed, Cu-NPs presented a higher potential for the production of somatic embryos compared to the plantlets/explant treated with $CuSO_4 \cdot 5H_2O$ (Ibrahim et al. 2019). Notably, ZnO NPs might positively increase the callus and somatic embryo induction (Devasia et al. 2020). In addition, the treatment of rhizome of Panax vietnamensis with Ag NPs could intensely induce somatic embryogenesis and plantlets (Du et al. 2021) (Fig. 14.5a, b).

The use of Phyto molecule-coated *Ulva lactuca* silver NPs (ULAgNPs) could also induce somatic embryogenesis and plant regeneration capacity in the rhizome explants of *Gloriosa superba*. Similarly, Ag NPs could efficiently enhance the percentage of somatic embryogenesis (almost 40%) in the explants of *Begonia tuberousvia* through cell layer culture (Mahendran et al. 2018). Notably, Cu-NPs and also Fe_3O_4-NPs could significantly improve somatic embryogenesis in the explants of *Ocimum basilicum* (84%) and *L. usitatissimum* (100%) when compared to the

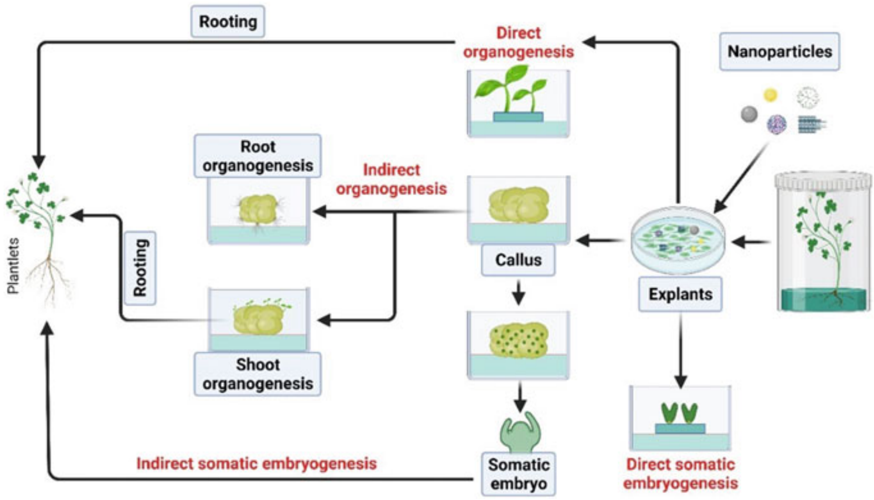

Fig. 14.4 effect of nanoparticles on organogenesis and somatic embryogenesis in plant tissue culture

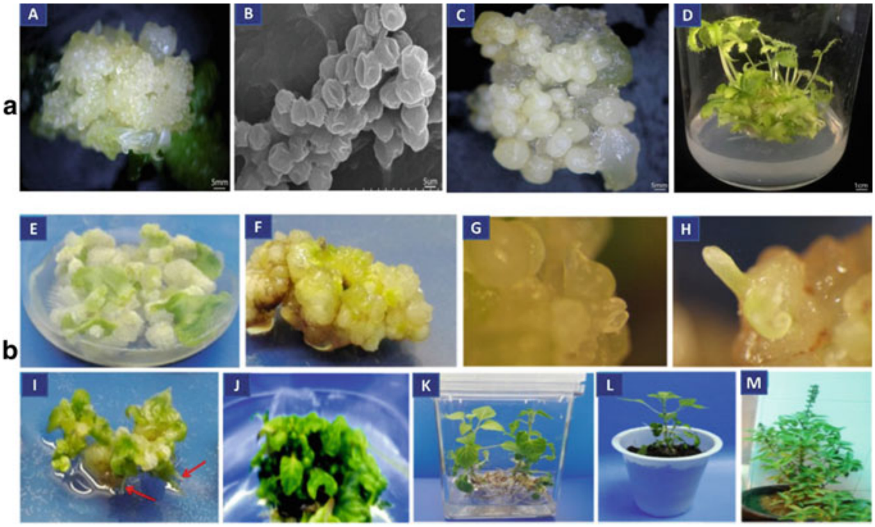

Fig. 14.5 The embryogenic calli induction and somatic embryo. *Panax vietnamensis* (**a**), *Ocimum basilicum* (**b**). *Source* (Du et al. 2021; Ibrahim et al. 2019)

control experiments (Ibrahim et al. 2019; Kokina et al. 2017a). However, the precise mechanism of NPs in somatic embryogenesis has not been understood yet, but these molecules might implicate their impacts by modulating the expression of some genes involved in embryogenesis (Kim et al. 2017).

14.5.4 Disinfection

Many NPs could be potentially utilized in superficial disinfection processes in the callus and cell suspension cultures (Sarmast and Salehi 2016). For instance, Ag NPs are effective in significantly decreasing bacterial contamination in the callus of *Vanilla planifolia* (Spinoso-Castillo et al. 2017). Moreover, Au NPs have frequently been utilised as an antimicrobial factor to surface sterilization of callus and explants in tissue culture experiments. The antibacterial, antiviral, antifungal, and antiseptic features of Au NPs have been relied on their potential to attack the wide range of organic processes in microorganisms inducing the disruption of the structure of plasma and cell membranes. These processes could lead to the depletion of intracellular ATP and cell death (Rudramurthy et al. 2016). Interestingly, plant-derived Au NPs could provide a better antimicrobial activity compared to the other NPs synthesized by physical and/or chemical methods. In detail, silver NPs are rapidly and environmental-friendly synthesized through the reduction of aqueous Ag^+ ions using *Dioscorea bulbifera* tuber extracts. The quality of the green AgNPs was evaluated by different approaches such as ultraviolet–visible absorption spectroscopy, high-resolution, and x-ray diffraction. Further data identified that this nanoparticle presented a potent antibacterial property against both gram-positive and gram-negative bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Ghosh et al. 2012).

14.5.5 Genetic Fidelity and Regeneration

Silver nano-complexes have positive impacts on the shoot regeneration capacity and genetic fidelity of in vitro-propagated *Alternanthera sessilis* cells. As a mutagenic factor, NPs could be efficacious for the induction of genotoxic effects in many plants because of their ease of interaction with plant cells (Kulus et al. 2022). Until now, the mutagenicity of ZnONPs and AgNPs was respectively confirmed in the *Allium cepa* and *Chrysanthemum* species (Kumari et al. 2011; Tymoszuk and Kulus 2020). The addition of AuNPs into the medium of *Lamprocapnos spectabilis* explants induced mutation in its genome which was detected by several molecular markers such as RAPD, SCoT, and DAMD markers (Kulus et al. 2022). These mutations mediated by NPs might result in phenotype and physiological variations in plants leading to the creation of new variants with improved characteristics. Moreover, the use of Phyto molecule-loaded silver nano-complex with AdS combination highly increases multiple shoot regeneration capacity in the *A. sessilis* cells (Venkatachalam et al. 2017). It is believed that many NPs especially Ag-related NPs have presented positive impacts on the improvement of regeneration capacity of different plant cell and callus cultures. In fact, NPs could downregulate several genes such as

1-aminocyclopropane-1-carboxylic acid (ACC) and 2-chloroethyl phosphonic acid (CEPA) to induce the pant regeneration pathways (Helaly et al. 2014). Moreover, the supplement of several plant cells such as tobacco, triticale, rape, and wheat with the increasing concentration of $CuSO_4$ NPs could improve the regeneration capacity of shoots (Purnhauser and Gyulai 1993). In addition, regeneration capacity through somatic embryogenesis in different recalcitrant cereal plants (e.g., barley, bread wheat, durum wheat, and rice) were enhanced upon treatment with a suitable concentration of CuNPs (Ibrahim 2012; Ibrahim et al. 2010; Eudes et al. 2003; Fahmy et al. 2012). It is also reported that CuO-NPs could significantly improve callogenesis and regeneration in the *Oryza sativa*. The suitable concentrations for improvement of regeneration capacity and callogenesis were identified as 20 mg/L and 10 mg/L of CuO-NPs (Anwaar et al. 2016).

14.6 Conclusions and Prospects

Today, nanotechnology has highly implicated in many industries especially agriculture, medicine, pharmacology, cosmetics, and environmental conservation. Different NPs have contributed to different aspects of plant biology including orogenesis, embryogenesis, tissue formation, differentiation, and development of plant cells and calli (Fig. 14.6). Notably, NPs especially are involved in the induction of secondary metabolites production and several pharmaceutical components through the up- or down-regulation of some plant genes. Moreover, plant cells and tissues could be considered as a more powerful platform for the production of different green NPs. However, further utmost research is needed to highlight the possible adverse effects of NPs on plant cell and tissue cultures. Plant cell and culture technology could be used as a green bio factory for the production of different valuable NPs. Notably, these green synthetized NPs could be regarded as a more powerful platform for drug delivery approaches provided fewer side effects.

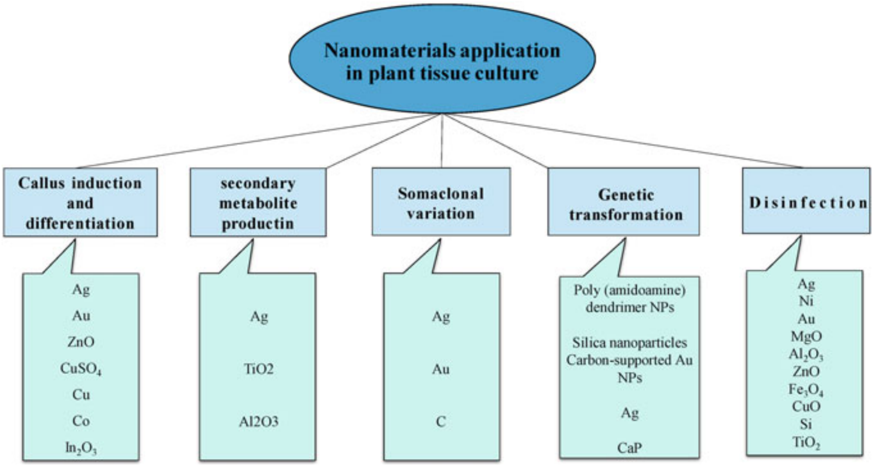

Fig. 14.6 Application of nanoparticles (NPs) in different aspects of plant cell and tissue culture approaches

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Chapter 15
Role of Nanomaterials in Improving the Nutritional Value of Crops

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Abstract In recent years, nanotechnology has been applied to revolutionize the technological progress in biomedicine, industry, and agriculture. Chemical, biological, and physical methods used for synthesis of nanomaterials substances and these materials applied at a tiny scale. The purpose of using nanomaterials in food and agriculture is to reduce the use of chemicals and nutrient losses and to increase crop yield through pest and nutrient management. In addition, these materials use to improve the nutritional value of crops. Due to the low macronutrient use efficiency and cost-intensive of conventional fertilizers, Nanomaterials are more effective than conventional fertilizers for the improvement of nutrient use efficiency. Nanomaterials are used to provide essential elements for plants directly or increment the efficiency in using nutrients. One of the main challenges facing developed and developing countries is the increasing demand for adequate and safe food and the risk of environmental damage by conventional agriculture. One way to boost agricultural efficiency is the use of nanotechnology. At present, numerous novel nanomaterials are commercially used in agriculture and have been developed to improve crop productivity and preserve food quality and safety. This chapter focuses on advances in nanotechnology and nanomaterials in improving the nutritional value of crops.

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15.1 Introduction

One of the most important parts of the industry in all countries in food industry. With the scarcity of food resources and the increase in population, the development of this part of the industry seems necessary. The use of new technologies in this area is a new approach that is highly regarded. Nanotechnology is one of the promising areas to enhance food production and to improve newer products for useful aims (Neme et al. 2021).

Nanomaterial (NMs) are engineered particles made to have their large surface–to-volume ratio and unique physical and chemical properties provide the clear advantage to use them in agriculture (Mittal et al. 2020). Several researchers have been widely studied the multiple types of nanomaterials, including fertilizers, herbicides, fungicides, pesticides, and sensors in crop production (Mittal et al. 2020).

Plants play an important role in human diet that provides carbohydrates, vitamins, minerals, proteins, lipids, fiber and water for our body (Goicoechea and Antolín 2017). Increasing the nutritional value of vegetables, fruits and other edible plants can improve the level of nutrients in plants without the need for increased consumption. There are several methods to significantly boost the nutritional levels of crops One of the methods is to use nanotechnology. Application of nanomaterials investigated for improving nutritional value in many crops. Several species have been exposed to different concentrations of nanomaterials and exposure to NMs significantly increased contents of carbohydrates, proteins, lipids, and antioxidant compounds in a plant (Gomez et al. 2021). In general, nanomaterials improved contents of nutrition characteristics in various plant via modulating nutrient concentrations, increasing chlorophyll content, enhanced photosynthetic activity, and enhancing key enzymes activity (Gomez et al. 2021).

There are many studies on the effects of nanomaterials on improving the productivity, protection, quality and postharvest decrements of many plants. However, no comprehensive review has yet been highlighted the impacts of different nanomaterials at diverse concentrations on improving the nutritional value of various crop plants. This present chapter includes literature concerning potential applications of nanomaterials in agricultural production and the effects of nanomaterials on food quality and improving the nutritional value of crops.

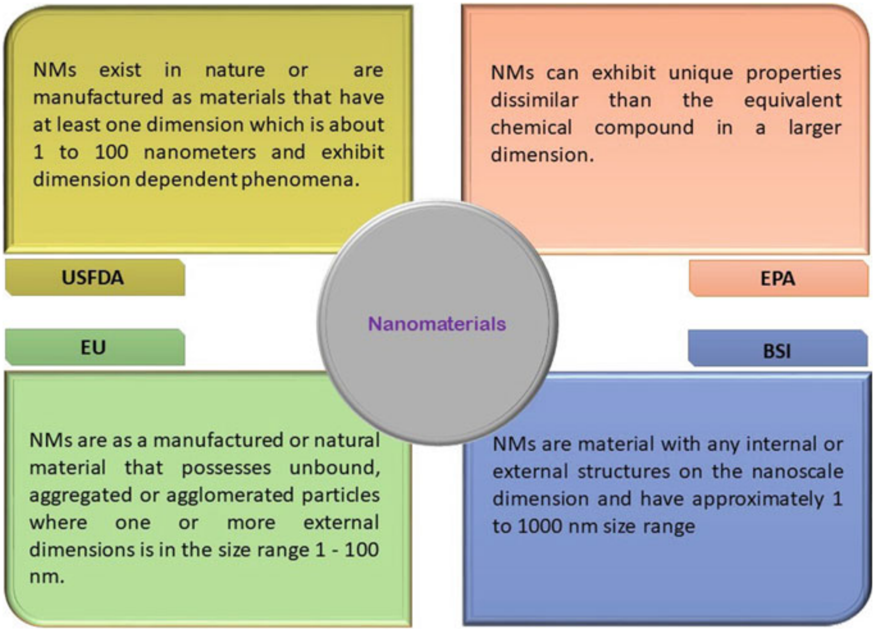


Fig. 15.1 Definition of nanomaterials as revealed by different organizations. EPA (Environmental Protection Agency), USFDA (United States Food and Drug Administration), EU (European Commission), BSI (British Standards Institution)

15.2 Nanomaterials Principles

15.2.1 Nanomaterials Definition

Nanomaterials (NMs) have mainly been used in every food industry, including food agriculture, processing, food storage, and transportation. In general, materials with at least one dimension with a length of 1 to 1000 nm are called nanomaterials. However, globally, nanomaterials are known as materials with diameters ranging from 1 to 100 nm (Jeevanandam et al. 2018).

There is no accepted cosmopolitan unit definition for NMs. Different organizations differ in the definition of NMs (Brandon et al. 2006), which are briefly discussed in Fig. 15.1.

15.2.2 Nanomaterials Synthesis

Nanomaterials have transpired as a phenomenal category of materials due to their unique electronic, optical, mechanical, magnetic, and chemical attributes compared

Fig. 15.2 Schematic representation of ‘top-down’ and ‘bottom-up’ methods for synthesis of nanomaterials

with their bulk counterparts (Khan et al. 2019). Two procedures have been applied to made nanomaterials successfully. Two main methods for nanomaterials synthesis are “top-down” and “bottom-up” that explained in this chapter (Luther 2004).

15.2.3 Top-Down Method

Top-down synthesis techniques have been used for producing micron-sized particles when a source of energy is applied. The top-down method entails smashing the bulk material into minor construction or particles using physical processes including milling, grinding, and crushing. In general, the top-down approach implicates the mechanical methods to break/crushing of bulk materials into several parts to get tiny size nanoparticles (Fig. 15.2).

15.2.4 Bottom-Up Method

The synthesis of nanoparticles means bottom-up with the interaction of atom-by-atom, molecule-by-molecule and, cluster-by-cluster through a set of chemical reactions provided by the Method (Fig. 15.2). This method is considered more straightforward and more precise, so it is more frequently applied in synthesizing small nanoparticles (Habiba et al. 2014). The bottom-up approach is more advantageous than the top-down approach because it produces a uniform shape, size, well-distributed nanomaterials, less contamination, and fewer defects.

The bottom-up approach consists of chemical vapor deposition, the sol–gel, solvo thermal and hydrothermal, soft and hard template, and reverse micelle methods (Baig et al. 2021).

15.3 Classification of Nanomaterials

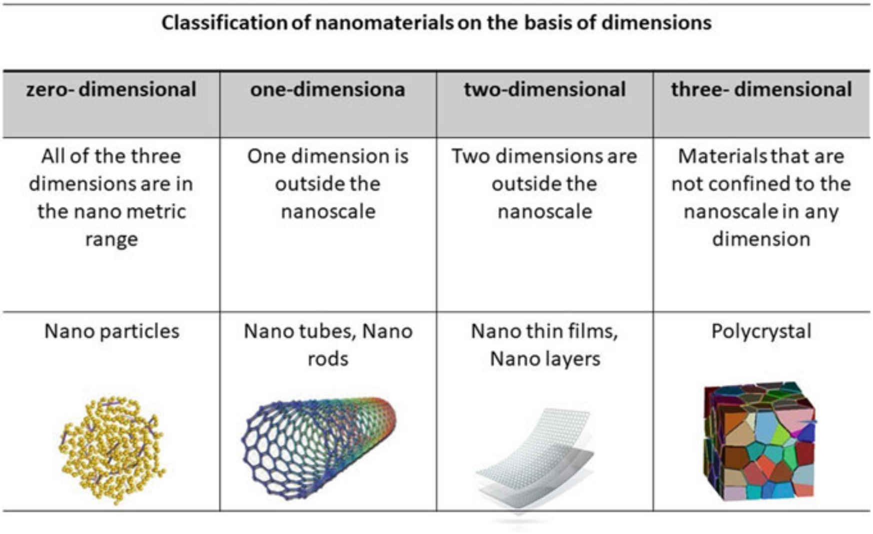
For variant use, several kinds of nanomaterials are made; it is required to classify these materials for appropriate usages (Khan 2020).

Nanomaterials are categorized according to their dimension of nanoparticles that are mainly classified into four types, including 0 Dimension (0D), 1 Dimension (1D), 2 Dimension (2D), and 3 Dimension (3D) nanomaterial. The nanomaterials classification is shown in Fig. 15.3, based on the number of dimensions.

On the other hand, nanomaterials can be classified as the natural origin or synthetically produced (Khan 2020).

Natural nanomaterials has made in microbes or plants through human activities and move annually between different compartments (atmosphere, biosphere, lithosphere, and hydrosphere) within the global biogeochemical cycle (Khan 2020; Lespes et al. 2020).

Synthetic Nanomaterials are mostly applied method to synthesis nanomaterials. Engineered nanoparticles synthetically play a significant role in nanotechnology applications. They have been used in many applications on a large scale,



Classification of nanomaterials on the basis of dimensions

zero- dimensional	one-dimensiona	two-dimensional	three- dimensional
All of the three dimensions are in the nano metric range	One dimension is outside the nanoscale	Two dimensions are outside the nanoscale	Materials that are not confined to the nanoscale in any dimension
Nano particles	Nano tubes, Nano rods	Nano thin films, Nano layers	Polycrystal

Fig. 15.3 Classification of nanomaterials according to dimensions

including embedded in a solid body (nanocomposites), distributed in fluids (dispersed ferrofluids), and dispersions in gases (e.g., as aerosols) (Raab et al. 2011).

15.4 Types of Nanomaterials

As the science of nanotechnology is advancing rapidly and is used in many different fields, many types of nanomaterials have been synthesized and it is necessary to classify all of these nanomaterials based on shape, size and chemical synthesis to distinguish them from each other (Khan 2020). Regarding their chemical compounds, nanomaterials could be classified into four types such as (1) Carbon-Based Materials, (2) Organic Based Materials, (3) inorganic-based nanomaterials, and (4) composite-based nanomaterials. Commonly, inorganic-based nanomaterials comprise various metal and metal oxide nanomaterials, brawny antibacterial factors (Loomba and Scarabelli 2013; Majhi and Yadav 2021). Inorganic nanoparticles are synthesized from inorganic elements (Ag, Ti, Au) (Fytianos et al. 2020).

The organic-based nanomaterials are organized from organic matter (Jain et al. 2014; Khan 2020). Carbon nanomaterials are a distinct class of nanomaterials, and a growing family of materials made up of carbon atoms. They are used in multi-disciplinary fields because of their unique morphology and multifaceted properties (Rizwan et al. 2021).

Composite-based nanomaterials are generated by a combination of two or more materials; therefore, these are hybrid materials. Nanocomposites are frequently prepared by chemically oriented synthetic methods (Sen 2020). Examples of each type of nanomaterials are shown in Fig. 15.4.

15.5 Use of Nanotechnology in Agriculture

In recent decades, nanotechnology has grown exponentially and the use of nanomaterials (NMs) has become increasingly widespread. There are various essential applications of nanomaterials in various fields, including biomedical, environmental, industrial, and food industries (Zaman et al. 2014) (Fig. 15.5).

Agriculture faces many limitations such as nutrient deficiencies and environmental pollution, and nanotechnology can improve agriculture industry (Shang et al. 2019). To date, several types of nanomaterials (often based on metal and carbon) have been used to improve plant growth and development (Agrawal and Rathore 2014).

It can be applied to all aspects of agriculture, including food packaging, food processing, nutraceuticals, and biomedicine. This technology also seems beneficial in improving minerals, vitamins, phytochemicals, antioxidants, nutritious oils in crops (Ravichandran 2010).

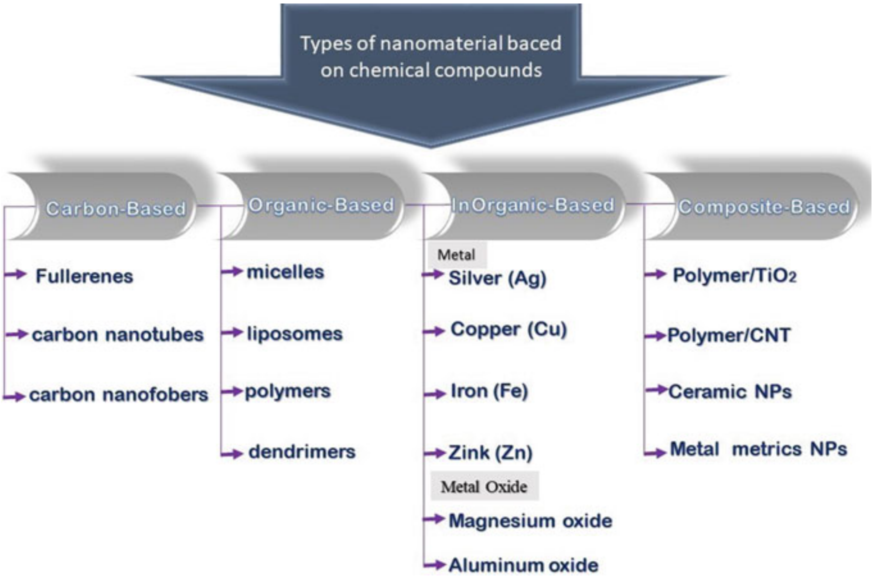


Fig. 15.4 Example of the type of nanomaterials based on composition

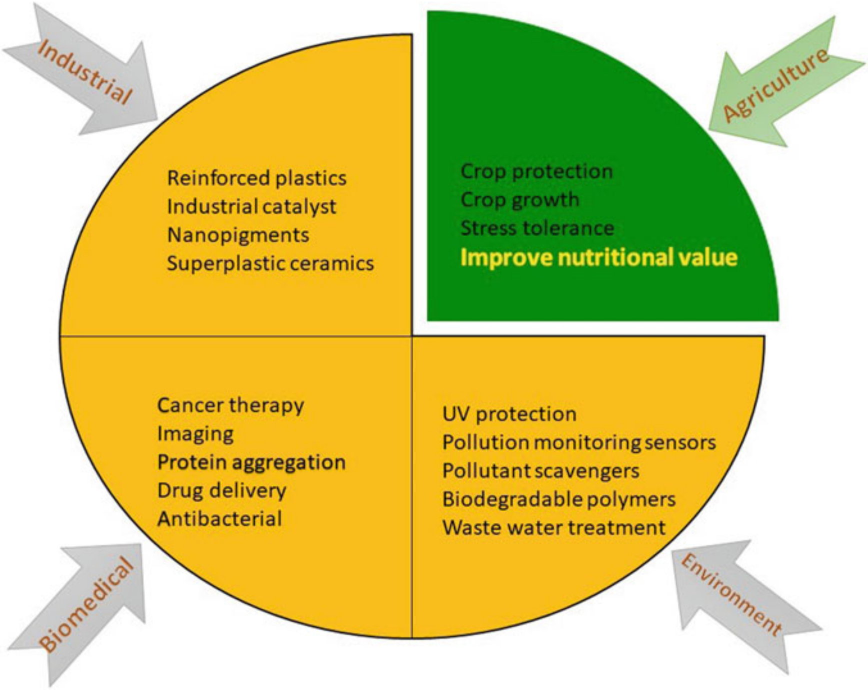


Fig. 15.5 Various applications of Nanomaterials

The objective of NMs applications in agriculture is to increase plant yields. Major plants provide the nutrients needed by humans and livestock. Functional food ingredients such as vitamins, phytochemicals, minerals, and antioxidants may exploit physiological effects beyond nutrition, promoting human health and disease prevention (Goicoechea and Antolín 2017). The use of nanomaterials improved the germination, development, and production of many crops. Tolerance to various biotic and abiotic stresses increased with the use of nanomaterials and also the application of nanomaterials improved the absorption of nutrients (Usman et al. 2020). These materials accomplish this through various mechanisms mentioned below (Liu et al. 2021).

Seed germination enhancement is induced through; (1) increased antioxidant enzyme activity and (2) improved assimilation and utilization of water and oxygen.

Growth improvement is induced through; (1) increased antioxidant enzyme activity, (2) improved plant cell morphology, (3) changes in the content of protein and organic compounds, (4) increased gene expression-related nutrient absorption, and (5) plant hormone balance.

Stress tolerance is induced through; (1) enhanced ability of scavenging ROS, (2) photosynthesis rate and photoprotection capacity, and (3) increased gene expression and protein abundance related to stress.

In common, at the low concentrations of nanoparticles has often been enhanced plant growth (root length, shoot length, biomass). Several pieces of research indicate high concentration of NMs cytotoxic for crops (Chenxu Liu et al. 2021; Rana et al. 2021). For example, AgNPs showed some toxicity at higher concentrations in poplars and Arabidopsis (Wang et al. 2013), squash onion (Liu et al. 2021), corn (Saha and Gupta 2017), and Common bean (Hediat MH Salama 2012).

The direct impact of toxicity of nanomaterials at the high doses includes inhibiting seed germination, decreasing leaf development and biomass production, and restricting root elongation (Lee et al. 2010; Colman et al. 2013; Yang et al. 2017).

The impact of nanomaterials on germination, growth, and plant protection is summarized and presented in Table 15.1.

15.6 Nanomaterials and Nutritional Value of Crops

The major nutrients required for the human diet supply from plants. The nutritional quality of crops affects human health. The progress of nanotechnology presented a new manner to increase the nutritional value of plant products, improve the rate and quality of carbohydrates, protein, and lipids in various plants and enhance amounts of antioxidants components (Fig. 15.6).

Although, there is considerable research into the relationship of nanomaterials to plants, but publications on the improvement of plant nutritional value by nanomaterials are yet limited (Wang et al. 2020).

Table 15.1 Impact of different nanomaterials on crop physiology and plant protection

Nanomaterial	Crop species	Dose used	Effect on plant	References
AgNPs	Wheat	50 mg/L and 75 mg/L	Improved growth and tolerance to heat stress	Iqbal et al. (2019)
AgNPs	Onion	5 and 10 µg/mL	promote growth without damage to roots or bulbs	Casillas-Figueroa et al. (2020)
AgNPs	Strawberry	0. 5 mg/L	Stimulates the growth of shoot and plantlet and shortened the duration of root formation in vitro	Tung et al. (2021)
AgNPs	Banana	1 and 5 mg/L	A significant increment in FW, DW and the number of roots	El-Mahdy et al. (2019)
AgNPs	Pearl millet	20 mM	Improved plant growth by reducing oxidative stress and Na and Cl absorption	Khan et al. (2021)
AgNPs	Brassica	50 ppm	Positive effect on root and shoot length and seedling index	Sharma et al. (2012)
Carbon nanotubes (MWCNT)	Tomato	10–40 mg/L	Enhances germination and growth rate but inhibits elongation of root	Khodakovskaya et al. (2013)
Carbon nanotubes (MWCNT)	Wheat, maize, peanut and garlic	50 µg/mL	Increased biomass accumulation and Improved and rapid germination,	Srivastava and Rao (2014)
Carbon nanotubes (MWCNT)	Onion and cucumber	10–40 mg/L	Enhances elongation of the root	Cañas et al. (2008)
Cu NPs	Chili	below 50 ppm	Promotes growth and height of a plant	Méndez-Argüello et al. (2016)
CuO	Spinach	200 mg/kg	Improved photosynthesis and biomass production	Wang et al. (2019)
Fe/SiO$_2$	Barely and corn	15 mg/kg	Enhanced plantsgrowth and biomass accumulation	Najafi Disfani et al. (2017)

(continued)

Table 15.1 (continued)

Nanomaterial	Crop species	Dose used	Effect on plant	References
Fe_3O_4	Bean	1000–2000 mg/L	Positive effect on nutrient uptake	De Souza et al. (2019)
Fe_3O_4	Corn	50 and 500 mg/kg	significantly increased the maize root length	Yan et al. (2020)
FeS_2	Carrot, alfalfa, mustard, sesamum	80–100 μg/mL	Increased germination and crop yield	Das et al.(2016)
TiO_2	Spinach	1000 to 2000 mg/L	Promotes growth and photosynthesis	Yang et al. (2006), Hong et al. (2005)
TiO_2	Wheat	2 and 10 ppm	Promotes seed germination	Feizi et al. (2012)
TiO_2	Fennel	60 ppm	Promotes seed germination and shoot dry weight	Feizi et al. (2013)
TiO_2 and SiO_2	Corn	20 and 30 mg/L	improved growth by stimulating antioxidant	Rizwan et al. (2019)
ZnO	Wheat	20 mg/L	Increased grain yield and biomass accumulation	Wei Du et al. (2019)
ZnO	Tobacco	0.2 μ M and 1 μ M	Promotes growth physiology of plants	Tirani et al. (2019)
ZnO	Mung bean	20, 40, 60 and 100 mg	Enhances germination rate, fresh and dry weights of roots and shoots	Jayarambabu et al. (2014)
ZnO	Soybean	0.05–0.5 mg/L	Affected photosystem II quantum efficiency	Priester et al. (2017)
ZnO and TiO_2	Tomato	0 to 1000 mg/ kg	Enhances growth and development	Raliya et al. (2016)
ZnO, CuO and AgNPs	Cluster bean	10 mg/L	Improved plant growth, biomass accumulation	Raliya and Tarafdar (2013)

15.6.1 Role of Nanomaterials in the Carbohydrate Content

Carbohydrates provided energy to all cells in the human body, and are one of the vital food nutrients. In plants, carbohydrates produced by photosynthesis activity exhibit the most significant proportion of organic compounds and because of their essential

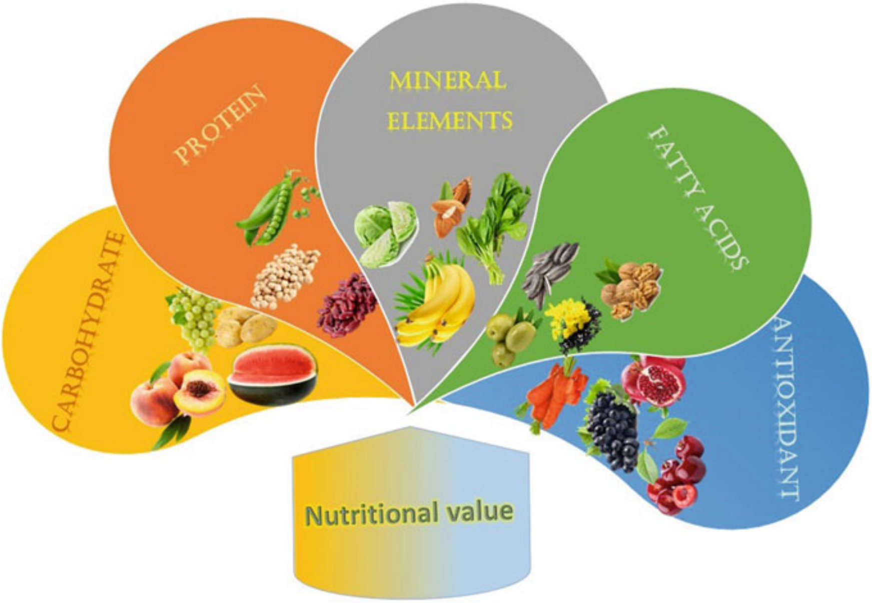


Fig. 15.6 Some indices of plant nutritional value

role in supplying energy, they are known as a vital source of energy (Trouvelot et al. 2014).

A significant increase in carbohydrates under silver nanoparticles (AgNPs) treatment was reported in *Pisum sativum* L. seeds (Mehmood and Murtaza 2017). Also, Salama (2012) reported that 600 ppm AgNPs enhanced the carbohydrate content of *Phaseolus vulgaris* L. and *Zea mays* L. plants by 57% and 62%, respectively. A remarkable decreasing (19 and 18% for common bean and 28 and 31% for maize relative to the control) in carbohydrate content was observed at 80 and 100 ppm AgNPs. Another study showed an increasing carbohydrate in plants by using ferric oxide nanomaterials (Liu et al. 2005). Total carbohydrate content in leaves of *Linum usitatissimum* L. by 54.15% over the control greatly increased by foliar application of ZnO nanoparticles (Singh et al. 2021).

Supplementary usage of zinc, boron, and zeolite nanoparticles in potato tubers significantly increased starch and carbohydrate content (Mahmoud et al. 2020). In general, nanomaterials have a critical impact in enhancing the total chlorophyll content, leave photosynthesis, and CO_2 assimilation, which raises the production of carbohydrates in the economic parts of crops (Song et al. 2014; Wang et al. 2015; De Smedt et al. 2017; Mahmoud et al. 2020). Also, nano-SiO_2 enhances carbonic anhydrase activity that supplies more carbon dioxide for the Rubisco, consequently improving the photosynthesis machine (Xie et al. 2011; Siddiqui and Al-Whaibi 2014).

Iron oxide nanoparticles enhance chlorophyll a and chlorophyll b content in soybean and improve the biochemical reactions of thylakoid membranes and chloroplast that improve the plant's photosynthetic activity and carbohydrates biosynthesis (Mohammadi 2015).

15.6.2 Role of Nanomaterials in the Protein Content

Proteins are the fundamental macronutrients, which our body needs in larger amounts. Plant foods can be great origins of protein. They are polymers with a complex three-dimensional structure that are made up of different amino acids and are required for chemical processes and general metabolism in living cells (Kinsella 1982). Many efforts made to improve protein content in plants. One of these procedures is the usage of nanomaterials in crops. Several studies suggested that nanomaterials could improve the content of protein in crops.

Nanosilver can interact with protein production (Krishnaraj et al. 2012). The maximum protein content was found for *Pisum sativum* L. seeds treated with 60 ppm AgNPs (Mehmood and Murtaza 2017). Also, the highest protein contents (30% for common bean and 24% for corn) in response to AgNPs application at 60 ppm have been studied in *Phaseolus vulgaris* L. and *Zea mays* L. (Salama 2012). The results of (Liu et al. 2005) indicated that iron oxide nanomaterials also significantly increased protein content in plants. Total protein of leaves for *Zea mays* L., *Phaseolus vulgaris* L., *Hyssopus officinalis* L., *Nigella sativa* L., *Amaranthus retroflexus* L. and *Taraxacum officinale* L. 400 mg L^{-1} of SiO_2 NPs showed a significant increase relative to the control (Sharifi-Rad et al. 2018). Mahmoud et al. (2020) showed enhancement of protein content in potato tubers treated with zinc, boron, and zeolite nanoparticles. It has been demonstrated that zinc, boron, and zeolite nanoparticles contribute to multiple enzymes activation involved in protein production (Wang et al. 2013).

WsCNOs (bio-waste derived) exposed *Cicer arietinum* L. plants produce seeds that exhibited a notable enhancement in their yield and increased protein content in the seeds (Tripathi et al. 2017). Raliya and Tarafdar (2013) showed in Cluster bean (*Cyamopsis tetragonoloba* L.), that total soluble leaf protein was increased by 27.1% with 10 mg/L ZnO treatment. Studies also revealed that the use of Silver nanoparticles (AgNPs) (100, 500, and 1000 mg/L) boosted protein content in *Brassica juncea* L. (Indian Mustard) at all doses (Pandey et al. 2014).

Application of CeO_2 at 100 and 400 mg/kg on wheat plants increased grain protein by 24.8% and 32.6%, respectively (Du et al. 2015). Also, there was an increment in protein levels of the *C. arietinum* seeds, by using an aqueous colloidal dispersion of copper nanoparticle-grown carbon nanofiber (Cu-CNF) (Ashfaq et al. 2017).

Higher protein contents were found in Bell Pepper under treatment of ZnO nanoparticles (Uresti-Porras et al. 2021). In addition, zinc oxide nanoparticles have been shown to act as a regulatory cofactor in protein biosynthesis in crops. In spinach, has been upregulated contents of amino acids such as methionine, cysteine, asparagine, and glutamine by using CeO_2 ENPs (0, 0.3, and 3 mg per plant) (Zhang et al. 2019). Similarly, use of ZnO NPs at 1000 mg/L enhanced protein content in leaves of spinach (Kisan et al. 2015). Also, CeO_2 ENPs at 125 mg/kg significantly increased linolenic acid by 6.17% in wheat grains (Rico et al. 2014).

Another study also showed that treatment of the *Linum usitatissimum* L. plant with nano TiO_2 at 100 mg/L increased the protein content (19.8 and 22.28%) (Aghdam et al. 2016). Yang et al. (2006) demonstrated that the use of TiO_2 NPs pronouncedly raised the activities of glutamate dehydrogenase, nitrate reductase, glutamic-pyruvic transaminase, glutamate dehydrogenase, and glutamine synthase in plants. Nitrate uptake and conversion of mineral nitrogen to organic nitrogen compounds such as protein and chlorophyll increased by TiO_2 nanoparticles (Khater 2015). In general, glutamate dehydrogenase and glutamine synthetase are two key enzymes in the synthesis of amino acids in plants.

15.6.3 Role of Nanomaterials in the Mineral Elements

Both humans and plants need macronutrients and micronutrients for optimal growth and development. Minerals as nutrients are essential for boosting the metabolism and other biological activities of many organisms. Crops contain a wide range of minerals including potassium (K^+), iron (Fe^{2+}), calcium (Cu^{2+}), phosphorus (P), magnesium (Mg^{2+}), Zinc (Zn), Copper (Cu) and Manganese (Mn). A large number of studies demonstrated that NPs significantly have the benefit of high activity, stability, and productiveness in nutrient delivery.

Mahmoud et al. (2020) showed an increment in the tissue concentration of N, P, Ca, Zn and B of potato under nanoparticles treatment. Scientists have reported that nano-zinc enhances P absorption. It is demonstrated that nano-ZnO particles act as a cofactor and increase the activity of the phosphatase enzyme, also phytase, in mung bean (Raliya et al. 2016). Also, Si enhanced the bioavailability of phosphorus that may be due to the chemical competition for the adsorption sites between the silicate anions and dihydrogen phosphate (Liang 1999). According to Raliya and Tarafdar (2013), the use of 10 mg/L ZnO nanoparticles improved the P concentration by 10.8% in Cluster bean (*Cyamopsis tetragonoloba* L.).

The usage of the CNMs in proper concentration benefits mineral improvement (Verma et al. 2019). Application of wsCNOs increased plant growth, fruit yield and enhanced Manganese, Molybdenum, Copper, Zinc, Iron, and Nickel) content in *C. arietinum* seeds (Tripathi et al. 2017). Salama et al. (2019) reported foliar spray of 40 ppmZnO NPs on common dry bean (*Phaseolus vulgaris* L.) statistically increased contents of Nitrogen, Iron, and Zinc in leaves and seeds.

In Bell peppers, ZnO nanoparticles at 30 mg/L enhanced the N and P contents by 12.3% and 25.9% higher than the control plants. Also, the Mg^{2+}, Mn^{2+}, $Zn^{2+}$and Fe^{2+} contents were 36.8, 42.2, 27 and 45% higher compared to the control (Uresti-Porras et al. 2021). Dimkpa et al. (2017) showed an increment of N and Mg in sorghum by 8% and 16% under ZnO NPs treatments.

Srivastava and Rao (2014) concluded that presence of FeS_2 ENPs (80 μg/mL, 14 h) enhanced the levels of Calcium (36.6%), Zinc (58.0%), and Manganese (26.4%), compared to control. In coriander (*Coriandrum sativum* L.), contents of Ca, Fe, K, Mg, Mn, and Zn enhanced in application of 50 and 100 mg/L TiO_2 ENMs in a hydroponic system (Hu et al. 2020). ZnO NPs application also enhanced Zinc content in wheat (Zhang et al. 2017).

Rico et al. (2014) showed that use of 250 mg/kg CeO_2 ENM, resulted in outstanding enhancement in P, Mg, K, Ca, S, Zn, Cu, Fe, and Al contents of barley. Also, Zinc and Manganese content was increased by application of 500 mg/kg of TiO_2 ENMs. According to Servin et al. (2013) TiO_2 treatments at 500 mg/kg indicated an enhancement in K^+ (35%) and P (34%) contents of cucumber fruit. Treatment with 50 and 100 mg/kg CeO_2 NPs increased Molybdenum content in cucumber (Hong et al. 2016).

15.6.4 Role of Nanomaterials in the Antioxidant Capacity

Antioxidants are essential for normal plant functioning and can delay or inhibit the oxidation of lipids or other molecules. Carotenoids, vitamin C, E, K, Flavonoids and Phenolic Acids are some non-enzymatic antioxidants in plants (Carocho and Ferreira 2013) (Fig. 15.7).

In tomatoes, nano Zn increased the antioxidant capacity and improved photosynthetic efficiency (Faizan et al. 2018). In radish tubers, 250 mg/kg $nCeO_2$ enhanced antioxidant activity (Corral-Diaz et al. 2014). In *Phaseolus vulgaris* L. of Toska cultivar, application of nano-Gro experimented and an increase of total phenolic compounds and subsequently elevation of antioxidant capacity was observed under nano-Gro treatment (Kocira et al. 2015). Similar results were reported that applying nano-selenium in sweet basil (*Ocimum basilicum* L.) increased the antioxidant potential (Ardebili et al. 2015). Nanoparticles of selenium have a potential antioxidant and reported an improvement in the total antioxidant of groundnut plants by using nano-selenium (Hussein et al. 2019).

In the last years, enhanced concern use of the natural phenolic compounds that are divided into several subgroups, including phenolic acids, lignans, quinones, tannins, flavonoids, coumarins, curcuminoids, and stilbens (Giada 2013). They are well known for their health advantage related to antioxidant activity (Martillanes et al. 2017). Some researchers studied increasing phenolic compounds in various crops. Many researchers showed that nanomaterials could be used as enhancers of secondary metabolite synthesis including phenolic compounds in plants.

It has been reported that total phenolic compounds and anthocyanins in licorice seedlings by application of nanoZnO and CuO (Oloumi et al. 2015). In *Capsicum annuum* L. use of ZnO-NPs in a range of 100 to 500 ppm significantly enhanced total phenols, condensed tannins, and flavonoids (Casillas-Figueroa et al. 2020). An increment in total phenols of potato plants by 1, 20 and 22%, also was observed at 100, 300 and 500 ppm ZnO-NPs treatment, respectively (Raigond et al. 2017). Under

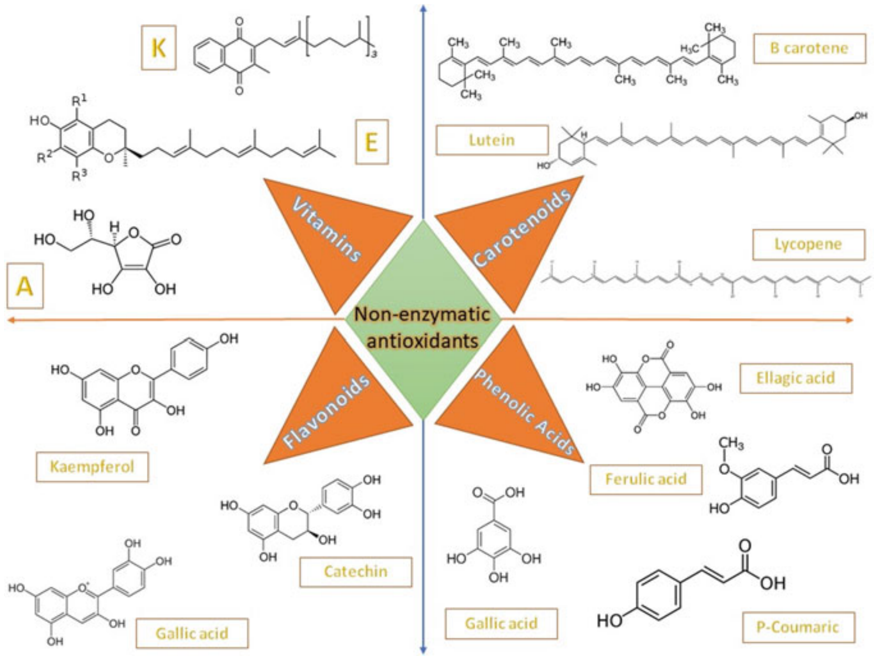


Fig. 15.7 Types of non-enzymatic antioxidants

use of ZnO-nanoparticles on *Brassica nigra* L. was also obtained an increment in phenolic compounds (Zafar et al. 2016). Phenolics and flavonoids constituents are synthesized via the shikimate phenyl propanoids-flavonoids pathway and zinc plays a role in the application of carbon to produce phenolic compounds via this pathway (Misra et al. 2006).

In addition, exposure to 100 μg/mL MWCNTs (multi-walled carbon nanotubes) extremely improved two important phenolic acids (caffeic acid and rosmarinic acid) contents in *Satureja khuzestanica* L. in in vitro condition (Ghorbanpour and Hadian 2015). In another study, under different concentrations of TiO_2NPs showed a substantial gain in total phenolics of leaf and flavonoids contents of *Salvia officinalis* L. In general, following the enhanced H_2O_2 content caused by the use of high levels of MWCNTs, significantly increased contents of phenolics, rosmarinic acid, flavonoids, and caffeic acid (Ghorbanpour 2015).

Use of ZnO NPs (80 mg/L) improved the phenolic content of rapeseed seeds (Akhavan Hezaveh et al. 2020). The total phenolic content (TPC) of rice grains is enhanced with nano fertilizer (FRR-NF) (Benzon et al. 2015). Although, other studies showed that the zinc and boron nano-fertilizers statistically enhanced total phenolic compounds in pomegranates (*Punica granatum* cv. Ardestani) fruit (Davarpanah et al. 2016).

Another studyobserved that flavonoid content (23%) and (44%) in the canola varieties Faisal canola and Shiralee, respectively increased by using 15 mg/L and 25 mg/L Zn nanoparticles (Kamran et al. 2020).

15.6.5 Role of Nanomaterials in the Fatty Acids Contents

Fatty acids are effective for normal growth and have therapeutic properties that prevent persistent illnesses. One of the important use of some plant species includes the extraction of oil from different plant tissue. Many authors reported that nanomaterials alter the content of Fatty acid in various plants. Some of the main fatty acids in plant oil are palmitic, oleic, linolenic, linoleic, and stearic acids.

An increase in essential oil was obtained at 2000 ppm ZnONPs (0.9% V/W) in Feverfew (*Tanacetum parthenium* (L.) Schultz Bip.) (Shahhoseini et al. 2020). Kamran et al. (2020) exposed both canola varieties (Faisal canola and Shiralee) to Zn NPs and reported an increase in oleic acid and linolenic acid. Faisal canola at Zn NPs 25 mg/L had maximum oleic acid (68.4%) and variety Shiralee had maximum oleic acid (66.1%) at 5 mg/L Zn NPs. The maximum linolenic acid recorded 12.6% at 25 mg/L with treated seeds with Zn NPs andwas (9.5%) in control plants for Shiralee. Similarly, Rui et al. (2017) reported an increase in oleic acid by 49.1% in peanuts on treatment with TiO NPs. Also, peanut crop exposed to 500 mg/kg of Ag NPs had an increase in linolenic acid (Rui et al. 2018).

In another survey presented recorded a positive effect of nano-Se on the oil quality of the groundnut plants. Regarding Gregory var., nano-Se application enhanced palmitic and stearic saturated fatty acids at 20 ppm. On the other hand, oleic acid enhanced by 15.54 and 21.0% under treatment of 20 ppm and 40 ppm nano-Se respectively, compared to the control plants (Hussein et al. 2019).

Dragon's head (*Lallemantia iberica* Fisch. & C. A. Mey.) plants treated with NPKsoil + NPKnano + chelated-Fenano had the highest linolenic acid (65.362%), saturated fatty acids (9.54%), palmitic acid percentage (7.943%) (Mohammadghasemi et al. 2021). Soybean treated with nano-iron chelate fertilizer + farmyard manure showed an increase in oleic acid content (Mohammadi 2015). 40 mg/L silver nanoparticles improved linoleic acid content (58.58%) in *Helianthus annuus* L. (sunflower) and a minute increase (4.05%) in palmitic acid contents was recorded at a 20 mg/L of silver nanoparticles, while Oleic acid showed a 29% enhancement at 80 mg/L of Ag NPs (Batool et al. 2021).

15.7 Role of Nano-Encapsulation Technology in Improving the Nutritional Values of the Product

Accumulation of vitamins, minerals, and bioactive compounds in the edible tissues of plants can boost concentration and bio accessibility of these components. The great purpose of encapsulation is coating sensitive compounds with suitable wall materials and creating a delivery system for sensitive compounds into food formulations. Nanoencapsulation clearly could improve the nutritional values of the food product (Delshadi et al. 2020).

There are many wall materials for encapsulation of food components including Carbohydrates (Chitosan, Pectin, Alginates, Cyclodextrins, Cellulose derivates, and Modified starch), Proteins (Sodium caseinate, Soy proteins, Gelatin, Caseins, and Zein), Fat and Waxes (Bee wax, Lecithin and Hydrogenated vegetable oils) and Polymers (Polyglycolides, Polylactides, Polyorthoesters, Polycaprolactone, Polyacrylic acid, and Polyvinyl alcohol (Pateiro et al. 2021).

Some of the components, such as the lipophilic functional ingredients, including active lipids (omega-3 fatty acids) and oil-soluble flavors, vitamins, and nutraceuticals successfully have been encapsulated (Karunaratne et al. 2017). Loading of ascorbic acid into ionic gelation nanoparticles and TPP crosslinking stabilized ascorbic acid to heat during heat processing.

Rosemary extract antimicrobial activity improved by chitosan nano encapsulated in composition with γ-poly glutamic. Lee et al. (2019) reported that the use of an electrospinning method for carotenoids encapsulation extracted from tomato peel into zein nanofibers is an effective method to improve its antioxidant activity, thermal and storage stability (Horuz and Belibağlı 2019). Other researchers applied a core–shell nanofiber structure (zein-tragacanth) via coaxial electrospinning technique for encapsulation of aqueous saffron extract showed high thermal stability (Dehcheshmeh and Fathi 2019).

In general, novel nano-encapsulation technologies improve the stability and aqueous solubility of insoluble bioactive compounds and also provide a controlled release to protect their pharmacological/biological activity in the body.

15.8 Conclusion and Prospects

More recently, interest in the development of nanotechnology increased and the use of nanomaterials in plant science. Nanotechnology in agriculture is considered as one of the effective methods to greatly raise crop produce and feed world growing population. Therefore, due to population increase and the consequent increase in demand for food, there is a need to produce plants with higher nutritional value. Research has shown that one of the efficient approaches to improve the nutritional value of plants is the use of nanomaterials that can increase the nutritional value in plants by affecting the physiological and biochemical properties of plants. Although, more study is needed to investigate the toxicity effects of nanomaterial accumulation or deposition on plant tissues. In addition, it is necessary to study the impact of these materials in improving the nutritional value of crops in real soil conditions and at high cultivation levels.

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Chapter 16
Role of Nanomaterials in Improving Crop Tolerance to Abiotic Stress

Farhat Yasmeen

Abstract The nanomaterials include the particles of hundred nanometers are applied in various areas of physio-biological sciences with diverse range of effects. However, the mode of synthesis is the major determent for the properties of nanoparticles. The particles synthesized through physical and chemical methods have comparatively different effects than the nanoparticles synthesized through green methods. The diverse effects of nanoparticles also reduced on green synthesis. So, nanoparticles synthesized via green methods have wide range of utilization in almost every field of life i.e. agriculture, engineering, cosmetics industry as well as into the medicine. These varied fields of utilizations of nanoparticles led their way out into the environment. These nanoparticles challenged the plant cells with a varied inauspicious environmental situation that limit growth of plants and demarcated the production of cultivated flora where numerous abiotic stresses that include drought, salinity, temperature etc. already were impacting the yield and productivity of crops. The present chapter spotlight actual apprehension of plant responses to nanoparticles on morphophysiological responses of plants as well as change in stress response under nanoparticles application. The morpho-physiological effects and nanoparticles role in mitigating the various abiotic stresses are discussed in detail to apprehend the inexplicit mechanism of nanoparticles stress adaptation.

Keywords Nanotechnology · Nanoparticles · Crops · Morpho-physiology · Abiotic stresses

16.1 Introduction

Abiotic stresses and limited soil nutrients availability are serious environmental situations for decreasing physical and reproductive characters of plants (Gong et al. 2020). Being immobile nature of plants, they have to face all sort of abiotic stresses related to water, salt and temperatures (Zhang et al. 2022). Various mechanical perceptions

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have evolved within plants for these environmental challenges and send stress signals inter and intracellularly for survival and reproduction (Gong et al. 2020). However, these stressors negatively impact the plants distribution with decline in growth and productivity (Zhang et al. 2022). With uncomplimentary climatic variation and fast increase in population, there is a desire for more productive and stress-tolerant crops (Nowicka et al. 2018). At the same time, conventional crop improvement techniques have perhaps touched its limits, so; in this kind of scenario, nanotechnology could be considered as a possible solution.

The nanomaterials are characterized as the units with a size of less than hundred nanometer (Khot et al. 2012). The progress in nanotechnology expanded the utilization of nanomaterials in various areas of physio-chemical and life sciences (Roco 2001). Nanobiotechnology is a ghastly flourishing area of studies that utilizes biosynthetic and eco-friendly techniques which are known to be non-toxic, chemically stabilized, biocompatible and applied in wide range of cosmetics (Rosi et al. 2005).

The mode of synthesis is the major determent for the properties of nanoparticles. The style of synthesis includes variety of methods which could be physico-chemical and biological for the commercially crucial nanoparticles (Iravani et al. 2014). The physical methods of nanoparticles production include tube furnace, laser ablation, thermal dehydration and thermal decomposition (Yasmeen et al. 2020). However, in the case of chemical methods, chemical reduction using various reducing agents, electro-chemical techniques, physico-chemical reduction, and radiolysis are popular (Peyser et al. 2001). In the biological or green method, any part of living organisms e.g. bacteria, viruses and plants probably be consumed as capping and reduction agent (Yasmeen et al. 2020). The main objective is to understand the role of nanoparticles in the improvement of plants growth and productivity under various abiotic stress situations without impacting the natural environment.

16.2 Morpho-Physiological Impact of Nanomaterials on Plant

The highly precocious interdisciplinary agency with diverse potency in agriculture for enhancing crop production utilizing nanomaterials with different size, density and surface charge impacted the maturation and physical processes of various plant species (Ma et al. 2010). These nanoparticles with their morpho-physiological effects are enlisted in Table 16.1.

16.2.1 Alumina Nanoparticles

Alumina nanoparticles are most widely applied in defensive and marketable goods (Handy et al. 2008). Diverse utilization of alumina nanoparticles raised a concern

Table 16.1 Effects of nanomaterials on various morpho-physiological responses of crops

Nanomaterials	Crops	Morpho-Physiological response	References
Alumina nanoparticle	*Cucumis sativus* (cucumber)	Declined the growth of root	Yang and Watts (2005)
	Glycine max L. (soybean)	Elevated survival percentage and growth of root	Yasmeen et al. (2016a, b)
	Glycine max L. (soybean)	Elevated length of root	Mustafa et al. (2016)
	Raphanusraphanistrum	Enhanced the growth of root	Lin and Xing, (2007)
	Triticum aestivum L. (wheat)	Significant improvement in the length of root	Riahi-Madvar et al. (2012)
	Zea mays L. (maize)	Decline in elongation of root	Yang and Watts (2005)
	Zea mays L. (maize)	Stimulated length of root	Lin and Xing (2007)
Carbon nanoparticles	*Rosa indica* (rose)	Increase in the content of endogenous hormones	Hao et al. (2018)
	Allium cepa (onion)	Improved length of root	Canas et al. (2008)
	Curcurbita Pepo (pumpkin)	Declined overall plant weight	Stampoulis et al. (2009)
	Lactuca sativa (lettuce)	Declined length of root	Canas et al. (2008)
	Lycopersicon esculentum (tomato)	Improved germination rate and fresh biomass	Khodakovskaya et al. (2009)
	Lycopersicon esculentum (tomato)	Reduced root growth	Canas et al. (2008)
	Oryza sativa L. (rice)	Suspended flowering resulting in declined productivity	Lin et al. (2009)
	Triticum aestivum L. (wheat)	Elevated the length of root	Wang et al. (2012)
Cerium oxide nanoparticles	*Allium cepa* (onion)	Improved plant growth and nutrient content	Abd El-Aziz et al. (2019)
	Lactuca sativa (lettuce)	Stopped the growth of root	Ma et al. (2010)
	Lactuca sativa (lettuce)	Oxidative stress	Zhang and Sonnewald (2017)
	Lycopersicon esculentum (tomato)	Severe reduction in shoot growth	Lopez-Moreno et al. (2010)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Oryza sativa L. (rice)	Exponential rate of growth	Divya et al. (2018)
	Sorghum bicolor L. (sorghum)	Increased pollen germination and seed yield per plant	Djanaguiraman et al. (2018)
	Triticum aestivum L. (wheat)	Risen the growth, shoot weight, and productivity	Rico et al. (2013)
	Triticum aestivum L. (wheat)	Reduction in the plant weight	Du et al. (2015)
	Zea mays L. (maize)	Improved the growth of root and stem	Lopez-Moreno et al. (2010)
	Zea mays L. (maize)	Significant improvement in the growth of root and stem	Lopez-Moreno et al. (2010)
	Zea mays L. (maize)	Severe decline in plant weight	Lopez-Moreno et al. (2010)
Copper nnanoparticles	*Arabidopsis thaliana* (Arabidopsis)	Increase in root length and fresh weights	Shaw and Hossain (2013)
	Cucurbita pepo (pumpkin)	Declined plant weight and growth of root	Stampoulis et al. (2009)
	Curcurbita pepo (pumpkin)	Decrease in plant weight	Stampoulis et al. (2009)
	Elodea Canadensis (Canadian waterweed)	Reduced turgidity and pigment content of leaves	Johnson et al. (2011)
	Lactuca sativa (lettuce)	Increase in rate of shoot and root	Shah and Belozerova (2009)
	Lactuca sativa (lettuce)	Enhanced the seedling growth	Shah and Belozerova (2009)
	Lactuca sativa (lettuce)	Improved rate of shoot and root	Shah and Belozerova (2009)
	Landoltia punctuata (duckweed)	Improved growth with elevated chlorophyll content	Shi et al. (2011)
	Hordeum sativum (barley)	Declined the concentration of plastoglobule and starch granule on chloroplast	Rajput et al. (2019)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	O*ryza sativa* L. (rice)	Decrease in the germination rate and plant weight	Shaw and Hossain (2013)
	Rosa indica L. (rose)	Change in concentration of endogenous hormones	Hao et al. (2018)
	Triticum aestivum L. (wheat)	Declined the growth of seedling	Musante and White (2012)
	Triticum aestivum L. (wheat)	Decrease in the growth of root and seedling	Lee et al. (2008)
	Triticum aestivum L. (wheat)	Increase in plant growth and weight with improved nutrient uptake	Noman et al. (2020)
	Triticum aestivum L. (wheat)	Inhibited growth and structure of the root	Tang et al. (2016)
	Triticum aestivum L. (Wheat)	Declined the growth of root and seedling	Lee et al. (2008)
	Triticum aestivum L. (Wheat)	Decline in germination percentage	Yasmeen et al. (2015)
	Triticum aestivum L. (Wheat)	Increase in weights and lengths of plant	Yasmeen et al. (2018)
	Triticum aestivum L. (Wheat)	Increase in s all grain parameters	Yasmeen et al. (2017)
	*Triticum aestivum*L.(Wheat)	Elevated plant height and biomass with enhanced nutrient	Noman et al. (2020)
	Vigna radiate (mung bean)	Decrease in the growth of seedling	Lee et al. (2008)
	Zea mays L. (maize)	Stopped the growth of seedling	Wang et al. (2012)
	Zea mays L. (maize)	Stopped root extension	Wang et al. (2012)
	Zea mays L. (maize)	Increase in the contents of anthocyanin, chlorophyll and carotenoid	Nguyen et al. (2021)
Gold nanoparticles	*Lactuca sativ*a (lettuce)	Enhance root elongation	Barrena et al. (2009)
Iron nanoparticles	*Arabidopsis thaliana* (Arabidopsis)	Increase in root length	Kim et al. (2014)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Arachis hypogaea (peanut)	Elevated the rate of germination and development of plant	Li et al. (2015)
	Cucumis sativus (cucumber)	inhibitory effects	Mushtaq (2011)
	Curcurbita mixta (pang)	No toxic effect	Zhu et al. (2008)
	Fragaria ananassa (strawberry)	Improved plant biomass with increase in photosynthetic pigments and total soluble carbohydrate	Mozafari et. al. (2019)
	Lactuca sativa (lettuce)	Inhibition in germination	Trujillo-Reyes et al. (2014)
	Linum usitatissimum (flaxseed)	Completely inhibited germination	El-Temsah and Joner, (2012)
	Lolium perenne L. (ryegrass)	Significant increase in antioxidant enzymes activities, and lipid peroxidation	Wang et al. (2011)
	Oryza sativa L. (rice)	Improved plant length and biomass with improved level of proline, glutathione and phyto-chelatins	Bidi et al. (2021)
	Oryza sativa L. (rice)	Increased biomass with improved photosynthetic potential and nutrient uptake	Ahmed et al. (2021)
	Spinacia oleracea L. (wheat)	Increase in plant growth with improved biomass and leaf surface index	Moghadam et al. (2012)
	Triticum aestivum L. (wheat)	Enhanced the rate of germination and development of plant	Feizi et al. (2013)
	Triticum aestivum L. (wheat)	Increase in biomass and plant length	Yasmeen et al. (2016a, b)
	Triticum aestivum L. (wheat)	Increase in grain related characters	Yasmeen et al. (2017)
	Triticum aestivum L. (wheat)	Improved germination	Yasmeen et al. (2015),

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Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Triticum aestivum L. (wheat)	Increase in plant height and spike length with improved chlorophyll content	Adrees et al. (2021)
	Triticum aestivum L. (wheat)	Increase in plant height with spike length and improved the biomass and productivity	Rizwan et al. (2019a, b)
	Triticum aestivum L. (wheat)	Increase in superoxide dismutase and peroxidase content	Konate et al. (2017)
	Triticum aestivum L. (wheat)	Increase in plant biomass with enhanced antioxidants and pigment content	Manzoor et al. (2021)
	Various plants	Development of thicker roots	Barrena et al. (2009)
	Vitis vinifera (grapes)	Significant increase in the total protein content, and antioxidant activities	Muzafari et al. (2018a; b)
Mixture of Au/Cu	*Lactuca sativa* (lettuce)	Enhanced rate of shoot and root	Shah and Belozerova (2009)
Mixture of SiO_2/TiO_2	*Glycine max L.* (soybean)	Increased germination and shoot growth with enhanced antioxidant system	Lu et al. (2002)
Nd_2O_3 nanoparticles	*Cucurbita pepo* (pumpkin)	Superoxide dismutase (SOD) and peroxidase (POD) activity increased, ascorbate peroxidase (APX) and catalase (CAT) decreased	Chen et al. (2016)
Nickel oxide nanomaterial	*LLycium barbarum* L. (boxthorn)	Overproduction of lipid peroxidation; proline, ascorbate, glutathione and phenols	Pinto et al. (2018)
Potassium silicate	*Vitis vinifera* (grapes)	Significant increase in the total protein content and antioxidant activities	Mozafari et al. (2018a, b)
Silicon nanoparticles	*Crataegusaronia* L. (hawthorn)	Increased plant biomass with improved the photosynthetic rate and stomatal conductance	Ashkavand et al. (2015)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Fragaria ananassa (strawberry)	Change in growth and yield parameters with improved relative water content, water use efficiency	Zahedi et al. (2020)
	Glycine max L. (soybean)	Improved seedling weight and chlorophyll	Li et al. (2020)
	Mangifera indica L. (mangos)	Increased leaf area with elevated nutrient uptake and antioxidant activities	Elsheery et al. (2020)
	Musa acuminate (banana)	Increased number and length of shoot with improved chlorophyll content and decreased electrolyte leakage and malondialdehyde content	Mahmoud et al. (2020)
	Oryza sativa L. (rice)	Improved yield	Hussain et al. (2020)
	Oryza sativa L. (rice)	Reduced metal uptake	Cui et al. (2017)
	Pisum sativum L. (Pea)	Improved the growth, increase in defense enzymes and nutrient uptake	Tripathi et al. (2015)
	Triticum aestivum L. (wheat)	Improvement in chlorophyll and reduced the malondialdehyde content	Khan et al. (2020)
	Triticum aestivum L. (wheat)	Improved seedling height and chlorophyll	Jiang et al. (2021)
Silver nanoparticle	*Arabidopsis thaliana* (arabidopsis)	Elevated the assemblage of reactive oxygen species	Syu et al. (2014)
	Brassica oleracea (cabbage)	Declined length of root	Pokhrel et al. (2013)
	Carum copticum (cumin)	Risen absorbance of nutrient with decreased requirement for fertilizer	Seghatoleslami et al. (2015)
	Cucurbita pepo (pumpkin)	Decline in plant weight	Stampoulis et al. (2009)
	Cucurbita pepo (pumpkin)	Decline in ranspiration ratio	Stampoulis et al. (2009)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Oryza sativa L. (rice)	Enhanced root elongation	Yang et al. (2018)
	Triticum aestivum L. (wheat)	Decline in percent germination	Yasmeen et al. (2015)
	Triticum aestivum L. (wheat)	Decline in seedling weight	Singh et al. (2015)
	Triticum aestivum L. (wheat)	Elevated antioxidative enzymes activities	Mohamed et al. (2017)
	Triticum aestivum L. (wheat)	Decrease in growth of seedling	Vannini et al. (2014)
	Vigna radiata (mung bean)	Reduction in biomass of plant	Singh et al. (2015)
	Vigna radiate (mung bean)	Organized the growth of seedling	Singh and Kumar (2015)
	Zea mays L. (maize)	Increase in length of root	Pokhrel et al. (2013)
Se	*Oryza sativa* L. (rice)	Improved yield	Hussain et al. (2020)
Titanium oxide nanoparticles	*Coriandrum sativum* L. (coriander)	Improved plant growth with increased antioxidants activities	Sardar et al. (2022)
	Cucumis sativus (cucumber)	Inhibited root elongation	Lin et al. (2007)
	Daucus carota (carrot)	Stopped root extension	Yang and Watts (2005)
	DDracocephalum Moldavica L. (dragonhead)	Improved phenolic substances	Kamalizadeh et al. (2019)
	Linum usitatissimum L. (flaxseed)	Capsule number increased in every plant with enhanced seed weight	Aghdam et al. (2016)
	Nicotiana tabacum (tobacco)	The emergence of the secondary roots	Cox et al. (2016)
	Rosa indica (rose)	Improved the endogenous hormones	Hao et al. (2018)
	Spinacia oleracea (spinach)	Heightened the growth	Hong et al. (2005)
	Spinacia oleracea (spinach)	Improvement in growth of plant	Yang et al. (2006)
	Spinacia oleracea (spinach)	Significant effect on plant growth	Zheng et al. (2005)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Spinacia oleracea (spinach)	Increase in length and weight of seedlings	Gao et al. (2006)
	Spinacia oleracea (spinach)	Improved biomass and chlorophyll	Zheng et al. (2005)
	Spinacia oleracea (spinach)	Increase in weight	Hong et al. (2005)
	Spinacia oleracea (spinach)	Stimulated the process of nitrogen fixation	Yang et al. (2007)
	Spinacia oleracea (spinach)	Enhanced absorbance of light and promoted fixation of carbon dioxide	Linglan et al. (2008)
	Spinacia oleracea (spinach)	Improved germination and shoot growth with enhanced antioxidant system	Lu et al. (2002)
	Triticum aestivum L. (wheat)	Elevated root length with no effect on germination and plant weight	Larue et al. (2012)
	Triticum aestivum L. (wheat)	Length of shoot enhanced	Rafique et al. (2014)
	Triticum aestivum L. (wheat)	Declined the plant overall weight	Jacob et al. (2013)
	Vicia narbonensis (narbon vetch)	Decrease in rate of germination and length of root	Castiglione et al. (2011)
	Vigna radiata L. (mung bean)	Increased length and weight of radicle with decline in free redical	Katiyar et al. (2020)
	Vitis vinifera (grapes)	Enhances the antioxidant capacity and phenolic content	Kőrösi et al. 2018
	Zea mays L. (maize)	Positive impact on germination and growth of seedling with antioxidants activities	Shah et al. (2021)
	Zea mays L. (maize)	Decrease in germination as well as reduction in root length	Castiglione et al. (2011)
	Zea mays L. (maize)	Declined hydraulic conductivity, leaf growth, and transpiration	Asli et al. (2009)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
Zinc oxide nanoparticles	*Glycine max L.* (soybean)	Elevated root length	Lopez-Moreno et al. (2010)
	Glycine max L. (soybean)	Enhanced the growth of root	Lopez-Moreno et al. (2010)
	Glycine max L. (soybean)	Improved root and shoot growth with raised antioxidative defense enzymes	Ahmad et al. (2020)
	Leucaena leucocephala (maxican tree)	Induced seedling growth with increased pigments and soluble proteins	Venkatachalam et al. (2017)
	Lolium perenne (ryegrass)	Reduced biomass with broken root tips	Lin and Xing, (2008)
	Lolium perenne (ryegrass)	Declined overall plant weight with broken root tips and cortical cells	Lin and Xing, (2007)
	LLycopersicon esculentum (tomato)	Heightened plant growth with improved chlorophyll and photosynthetic attributes	Faiza et al. (2021)
	Mangifera indica L. (mango)	Improved leaf area with the overall yield and fruit physiochemical character	Elsheery et al. (2008)
	Oryza sativa L. (rice)	Stimulated the growth of seedlings	Yan et al. (2021)
	Pleuroziumschreberi (Brid.) Mitt	Reduced the content of L-ascorbic acid	Motyka et al. (2019)
	Solanum melongena L. (eggplant)	Increase in the plant growth and production	Semida et al. (2021)
	Triticum aestivum L. (Wheat)	Declined the plant weight	Du et al. (2015)
	Triticum aestivum L. (Wheat)	Declined the plant weight	Jacob et al. (2013)
	Triticum aestivum L. (Wheat)	Improved growth and productivity with elevated chlorophyll	Adrees et al. (2021)
	Triticum aestivum L.(Wheat)	Induced plant growth and biomass	Rizwan et al. (2019a, b)
	Triticum aestivum L.(Wheat)	Delay in the panicle initiation time with rise in grain yield	Dimkpa et al. (2020)

(continued)

Table 16.1 (continued)

Nanomaterials	Crops	Morpho-Physiological response	References
	Zea mays L. (maize)	Significant reduction in growth of root	Lin and Xing, (2007)
	Zea mays L. (maize)	Stimulate plant growth with improved chlorophyll content and antioxidants	Rizwan et al. (2019a, b)
Zn-, B-, Si-, and Zeolite NPs	*Solanum tuberosum* L. (potato)	Increase in plant height and tuber yield	Mahmoud et al. (2019)

about their possible escape into environment with ultimate interaction with living organisms (Shabnam and Kim, 2018). Treatment of aluminum oxide nanoparticles stimulated the chances of survival with elevated weight and length of root in soybean (Yasmeen et al. 2016a, b; Mustafa et al. 2016) with significant improvement in the length of wheat root (Riahi-Madvar et al. 2012) and lettuce (Lin and Xing 2007). Nevertheless, length of root was reduced in cucumber on alumina nanoparticles exposure (Yang and Watts 2005).

16.2.2 Carbon Nanoparticles

Utilization of carbon nanotubes in delivery systems of various drugs into cell is the development of this era. This advancement increased the effort of scientist to observe for the consumption and transportation of these nanotubes within the plant (Rico et al. 2011). Morphological parameters such as germination rate was increased in tomato on carbon nanoparticles exposure (Khodakovskaya et al. 2009) with significant increase in root length of onion (Canas et al. 2008), and wheat (Wang et al. 2012). Carbon nanoparticles treatment also enhanced germination of seed with extended hypocotyl and larger cotyledon area leading to elevated chlorophyll content in Arabidopsis seedlings (Kumar et al. 2018). However, flowering was delayed and productivity was reduced in rice with carbon nanoparticles treatment (Lin et al. 2009). Carbon nanoparticles increased fungal resistance in rose plants by modifying the concentration of endogenous hormones (Hao et al. 2018).

16.2.3 Cerium Oxide Nanoparticles

Nanoceria are the utmost auspicious candidate for managing soft tissue lesions because of antioxidant, anti-inflammatory and antibacterial activities (Sadidi et al. 2020). The growth of the root was increased, whereas of shoot was inhibited in soybean (Li et al. 2020) and wheat (Rico et al. 2013). However, root growth was inhibited in lettuce (Ma et al. 2010). Significant reduction in biomass was observed in maize (Lopez-Moreno et al. 2010) and in wheat (Du et al. 2015). Nano ceria directly influence the process of photosynthesos by inhibiting chlorophyll synthesis and reducing the photochemical quenching in soybean (Li et al. 2020).

16.2.4 Copper Nanoparticles

Amongst the metalic nanomaterials, copper nanoparticles are utilized in electronics, wind and hydraulic filtration, ceramics, wood preservatives, and bioactive coverings, showbiz industries and textile, makeups, and in lubricants oil (Yang et al. 2009). These wide range utilizations has heightened the interest of their effect on overall ecosystem (Chen et al. 2010). Copper nanoparticles has altered the morphological characters in various plants such as elevation in the height of plant and shoot dry weight of wheat (Noman et al. 2020), improved rate of shoot and root in lettuce (Shah and Belozerova 2009), elevated the weights and lengths of wheat plant (Yasmeen et al. 2018). While; couple of studies also indicated negative response of copper nanoparticles such as decline in growth of wheat and mungbean (Lee et al. 2008), declined plant weight and growth of root in zucchini (Stampoulis et al. 2009), decreased the rate of germination and plant weight in rice and Arabidopssis (Shaw and Hossain, 2013), quenched root elongation in maize (Wang et al. 2012), and repressed development and assembly of the root in wheat (Tang et al. 2016).

Physiological responses were also altered on copper nanoparticles exposure such as treatment of copper nanoparticles stimulated the anthocyanin, chlorophyll, and carotenoid and elevated tolerance to water deficit by reducing the oxidative stress (Nguyen et al. 2021). Nutrient uptake was also enhanced on copper nanoparticles exposure in wheat (Noman et al. 2020). Compliance to the previous responses, copper nanoparticles could potentially be used as target agent for targeting any particular physiological response.

16.2.5 Iron Oxide Nanoparticles

Among the different metallic nanomaterials, iron nanoparticles are used in various commercialized enterprise, and biomedical activities to bring positive change in society (Teja et al. 2009). Due to higher reactivity and magnetic property, iron nanoparticles were exploited as rectification tools for ecosystem (Yan et al. 2013). Iron nanoparticles improved the morphological traits with increased productivity and grains in wheat (Yasmeen et al. 2017; Rizwan et al. 2019a, b; Manzoor et al. 2021; Adrees et al. 2021). Similarly, growth of rice was improved with the treatment of iron nanoparticles (Bidi et al. 2021; Ahmed et al. 2021). High concentration of iron nanoparticles increased dry weight of the explants of strawberry (Mozafari et al. 2019). However high concentration of iron nanoparticles was toxic to lettuce as germination was declined (Trujillo-Reyes et al. 2014). Other way round, stimulatory effects on the germination ratio and development of peanut (Li et al. 2015) and wheat (Feizi et al. 2013; Yasmeen et al. 2016a, b) were also discussed.

Utilization of iron nanoparticles raised the level of proline, glutathione and phytochelatins and defense enzymes in rice (Bidi et al. 2021) as well as in wheat (Konate et al. 2017), ryegrass and pumpkin (Wang et al. 2011). However, a noteworthy decline in proline content was observed in grapes (Muzafari et al. 2018a, b) and in strawberry (Mozafari et al. 2019). Iron nanoparticles increased depolluting enzymes, photosynthetic potential, and nutrient concentration while declined the formation of reactive oxygen species in rice (Ahmed et al. 2021). These studies explicit the response of iron nanoparticles is plant and concentration dependent for its positive or negative attribute.

16.2.6 Silicon Nanoparticles

After oxygen, silicon being the richest element; act as (Ma 2004) physico-mechanical barrier. Silicon is component of the epidermal cell walls and conducting tissues throughout the plant (Siddiqui et al. 2014). The stimulatory effects of the silicon macroparticles have been described in plants by researchers; though, nanoparticles of silicon are absorbed more comparative to bulks (Suriyaprabha et al. 2012). Morphological characters such as improvement in growth in pea (Tripathi et al. 2015), number and length of shoot in banana (Mahmoud et al. 2020), increased leaf area (in mango (Elsheery et al. 2020), enhanced the growth and productivity of strawberry (Zahedi et al. 2020), improved seedling fresh weight in soybean (Li et al. 2020) and improved yield in rice (Hussain et al. 2020).

Silicon with a size of 10 μM improved the growth, risen the synthesis of defense enzymes and increased nutrient uptake in pea (Tripathi et al. 2015). In wheat, treatment of silicon nanoparticles significantly elevated the chlorophyll contents and amend oxidative emphasis by declining the malondialdehyde, hydrogen peroxides and electrolyte leakage (Khan et al. 2020). All doses of silicon dioxide nanoparticles improved the growth of shoots in banana. Application of silicon dioxide nanoparticles in banana risen chlorophyll content, declined electrolyte leakage with the change in phenolic compound (Mahmoud et al. 2020). In the case of rice, silicon nanoparticles not only improved the yield (Hussain et al. 2020) but also edited the gene expression involved in declined metal uptake (Cui et al. 2017). These previous studies show that silicon nanoparticles could potentially be used as remediator in heavy metals prone areas.

16.2.7 Silver Nanoparticles

Being antibacterial in nature (Weir et al. 2008) silver nanoparticles are manufactured via various physico-chemical, and biological methods with specific size and shape (Iravani et al. 2014). Application of silver nanoparticles enhanced the reactions of critical antioxidative enzymes whilst decreased the concentration of stress signals in wheat leaves (Mohamed et al. 2017). Silver nanoparticles also decreased the growth in wheat (Vannini et al. 2014; Singh et al. 2015), zucchini (Stampoulis et al. 2009) and declined root length in mung bean (Singh et al. 2015), and in cabbage (Pokhrel et al. 2013) with reduced rate of germination in wheat (Yasmeen et al. 2015). However, root length was increased in maize on treatment with silver nanoparticles (Pokhrel et al. 2013).

Silver nanoparticles also enhanced the aggregates of reactive oxygen species in arabidopsis (Syu et al. 2014) and stimulated the activities of dynamic antioxidative enzymes whilst decreased the contents of malondialdehyd and hydrogen peroxide in wheat (Mohamed et al. 2017) with tremendous improvement of water use efficacy, nutrient uptake and decreased fertilizer necessity in cumin seeds (Seghatoleslami et al. 2015). Silver nanoparticles raised the seedling growth in mungbean (Singh and Kumar 2015). However, percent germination was reduced in wheat (Yasmeen et al. 2015). Silver nanoparticles also reduced the seedling weight in wheat (Singh et al. 2015) and decreased plant biomass in mungbean (Singh et al. 2015). These earlier studies indicated the undesirable influence of silver nanoparticles on physical characters of plants and need further exploration.

16.2.8 Titanium Oxide Nanoparticles

Titanium dioxide nanoparticles are mostly applied nanomaterials in the variety of consumer and agriculture products enabling their passage into the environment (Hou et al. 2019). Diverse utilization of titanium dioxide nanoparticles has been documented in private skincare product and water-treatment mediator (Riu et al. 2006; Tan et al. 2007). Priming of maize seed with titanium dioxide nanoparticles positively impacted the germination and seedling growth while reduced the mean emergence time (Shah et al. 2021). However; in the case of wheat, root length was increased but rate of germination and plant weight was not enhanced (Larue et al. 2012). The emergence of the secondary roots was observed in tobacco (Cox et al. 2016). Radicle length and biomass in mungbean was increased with nanoparticles application (Katiyar et al. 2020). Root elongation was inhibited in cucumber (Lin et al. 2007), carrot (Yang and Watts 2005), while an improvement in growth of spinach was recorded (Hong et al. 2005; Zheng et al. 2005; Yang et al. 2006). Capsules ration per plant enhanced in plants on treatment with titanium dioxide nanoparticles and increased the seed weight in linseed (Aghdam et al. 2016).

Treatment of titanium dioxide nanoparticles also enhanced the number of potassium ion, relative water content, contents of total phenolic and proline; stimulated antioxidants activities; andreducedsodium ion concentration, membrane electrolyte leakage,in maize (Shah et al. 2021). However, in mungbean, there was a reduction in the level of free radicals and lipid peroxidation (Katiyar et al. 2020). So, titanium dioxide nanoparticles could potentially be the source of stress tolerable in plants.

16.2.9 Zinc Oxide Nanoparticles

Utilization of zinc-oxide nanomaterials in various commercial applications with a possible way out into the ecosystem (Rajput et al. 2018). Influence of zinc oxide nanoparticles are plant dependent as in wheat there was reduction in the biomass (Du et al. 2015; Jacob et al. 2013) while improved in soybean (Lopez-Moreno et al. 2010). Exposure to zinc oxide nanoparticles decreased the plant weight, thin root tips and damaged epidermis and root cap in ryegrass (Lin and Xing 2008). While; growth of seedling was improved with zinc oxide nanoparticles treatments in rice (Yan et al. 2021).

Growth and productivity was enhanced on spraying of zinc oxide nanoparticles with stimulated chlorophyll content and the antioxidant activities in wheat (Adrees et al. 2021). However; in wild tamarind, pigments and soluble proteins were improved while peroxidase was reduced (Venkatachalam et al. 2017). Photosynthesis was also improved in soybean with healthy root and shoot growth on zinc oxide nanoparticles exposure (Ahmad et al. 2020). Spraying of zinc oxide nanoparticles improved the eggplant growth and productivity (Semida et al. 2021). The total yield and physiochemical properties in mango increased with wider leaf area on zinc oxide nanoparticles treatment (Elsheery et al. 2020). Similarly; application of these nanoparticles stimulated all macronutrients and antioxidants activities in mango (Elsheery et al. 2020).

16.2.10 Miscellaneous

Employment of individual and binary treatment of zeolite nanoparticles in tomatos enhanced plant elevation, shoot dry mass, number of stems per plant, and tuber yield (Mahmoud et al. 2019). Nanoparticles treatment stimulated relative water content and chlorophyll concentration with improved nutrients uptake in tomatoes (Mahmoud et al. 2019). Application of potassium silicate in grapes importantly enhanced the total protein content and activities of antioxidative enzymes while reduced proline content (Mahmoud et al. 2019). Submission of nickle oxide nanoparticles in wolfberry resulted in excessive production of hydrogen peroxide and superoxide anion with ultimate increase in lipid peroxidation, proline, ascorbate, glutathione and phenols levels (Pinto et al. 2018). Mixture of nanoparticles has also altered the growth and yield of various plants. Mixture of silicate and titanium oxide nanoparticles increased germination and shoot growth with elevated nitrate reductase activity and enhanced antioxidant system (Lu et al. 2002). However, mixture of Au/Cu has zero response towards the germination but rate of shoot and root of lettuce was improved (Shah and Belozerova 2009).

16.3 Nanomaterials Response Against Abiotic Stresses

In agriculture, the application of nanoparticles is thriving rapidly and analyses of their effects are done through variation in the germination ratio, growth and development (Siddiqui et al. 2015). The nanoparticles interact in a straight line or by chance release in the neighborhood of plants (Rico et al. 2011). These nanoparticles have also been utilized to mitigate the effects of various abiotic stresses (Table 16.2) (Fig. 16.1).

Table 16.2 Effects of various nanomaterials in response to abiotic stresses on economically important crops

Stress	Nanomaterials	Crops	Effects	References
cold	Titanium dioxide	Chickpea (*Cicer arietinum*)	Enhanced redox status of plants	Mohammadi et al. (2013)
Drought	Iron	Wheat (*Triticum aestivum* L.)	Improved plant elevation, spike size and dry weight with chlorophyll	Adrees et al. (2021)
	Selenium	Strawberry (*Fragaria ananassa*)	Improved the growth and yield with elevated water use efficiency	Zahedi et al. (2020)
	Silica	Strawberry (*Fragaria ananassa*)	Improved the growth and yield with increased water use efficiency and antioxidant activities	Zahedi et al. (2020)
	Silica	Mango (*Mangifera indica* L.)	Enhanced leaf area with increased productivity and fruit qualities with enhanced antioxidant activities	Elsheery et al. (2020)
	Silica	Wheat (*Triticum aestivum* L.)	Increase in shoot, root, and grain dry biomass with enhanced chlorophyll and antioxidants	Khan et al. (2020)
	Silica	Hawthorn (*Crataegus sp.*)	Improved plant overall weight and xylem water potential with elevated the photosynthetic rate and stomatal conductance	Ashkavand et al. (2015)
	Titanium dioxide	Flax(*Linum usitatissimum*L.)	Step-up carotenoids content and ameliorated cell membrane damage	Aghdam et al. (2016)
	Titanium dioxide	Dragonhead (*Dracocephalum moldavica*L.)	Increased certain beneficial phenolic substances	Kamalizadeh et al. (2019)
	Zero-valent copper	Maize (*Zea mays* L.)	Improved the biomass and stimulated total seed number and grain yield with increased contents of anthocyanin,chlorophyll, and carotenoid	Nguyen et al. (2021)
	Zinc Oxide	Mango (*Mangiferaindica*L.)	Enhanced leaf area with increased productivity and fruit qualities	Elsheery et al. (2020)

(continued)

Table 16.2 (continued)

Stress	Nanomaterials	Crops	Effects	References
	Zinc Oxide	Wheat (*Triticum aestivum* L.)	Late panicle initiation time with exponential grain yield	Dimkpa et al. (2020)
	Zinc Oxide	Eggplant (*Solanum melongena* L.)	Stimulated the plant growth and productivity with enhanced macro- and micronutrients' uptake andincreased relative water content	Semida et al. (2021)
	Zinc Oxide	Wheat (*Triticum aestivum* L.)	Improved growth and productivity with enhanced chlorophyll and antioxidants activities	Adrees et al. (2021)
Heavy metal stress	Gold	Rice (*Oryza sativa* L.)	Bettermet in antioxidant defense enzyme with limited manifestation of genes associated with metal transport	Jiang et al. (2021)
	Silica	Wheat (*Triticum aestivum* L.)	Improved chlorophyll and photosynthesis	Jiang et al. (2021)
	Zinc Oxide	Wheat (*Triticum aestivum* L.)	Evoked plant growth, dry weight, and grains and declined the loss of electrolyte and antioxidant activities	Rizwan et al. (2019a, b)
High temperature Stress	Silver	Wheat (*Triticum aestivum* L.)	Improve length of root and shoot with increased root number and plant biomass	Iqbal et al. (2017)
Salinity	Carbon	Lettuce (*Lactuca sativa*)	Significant improvement in the germination rate n some	Baz et al. (2020)
	Iron	Grapes(*Vitis vinifera*)	Significant increase in the total protein content with improved activities of antioxidative enzymes and hydrogen peroxide	Mozafari et al. (2018a, b)

(continued)

16.3.1 Drought

The utmost vital source for the survival of plants is water which is required for the transportation of nutrients which went under threat during the period of drought (Martinez-Vilata and Pinol 2002). Growth of plant declined (Bigler et al. 2006) with elevated rate of mortality (Rebetez and Dobbertin 2004). Under stress situations, the

Table 16.2 (continued)

Stress	Nanomaterials	Crops	Effects	References
	Iron	Strawberry (*Fragaria ananassa*)	Enhanced dry weight of the root and explants with improved photosynthetic pigments and total soluble carbohydrate, membrane stability index, and relative water content	Mozafari et al. (2019)
	Iron	Wheat (*Triticum aestivum* L.)	Elicited plant growth, dry weight, and grains and declined the loss of electrolyte and antioxidant activities	Rizwan et al. (2019a, b)
	N–Na2SiO3	Potato (*Solanum tuberosum* L.)	Increased tuber productivitywith increased water use efficiency and tuber dry matter percentage	Kafifi et al. (2019)
	Potassium silicate	Grapes(*Vitis vinifera*)	Significant increase in the total protein content, activities of antioxidative enzymes and hydrogen peroxide	Muzafari et al. (2018a; b)
	Silica	Banana (*Musa acuminata*)	Increased the nu Increase in number of shoots and shoot length with improved chlorophyll and lowered electrolyte leakage	Mahmoud et al. (2020)
	Silver	Wheat *Triticum aestivum* L.)	Stimulated the weight with increased the activities of vital antioxidative enzymes	Mohamed et al. (2017)
	Titanium dioxide	Maize (*Zea mays L.*)	Stimulated the germination and growth of seedling and reduced the mean emergence time	Shah et al. (2021)
	Zinc Oxide	Fenugreek (*Trigonella foenum-graecum*)	Interaction reverse the salinity evoked consequences	Noohpisheh et al. (2021)
	Zinc Oxide	*Tomato (Lycopersicon esculentum*)	Increase in length of shoot and root, weight, and leaf area with improved chlorophyll and photosynthetic attributes	Faizan et al. (2021)

(continued)

Table 16.2 (continued)

Stress	Nanomaterials	Crops	Effects	References
	Zn-, B-, Si	Potato (*Solanum tuberosum* L.)	Stimulated plant height, shoot dry weight, and tuber yield with increased photosynthetic rate	Mahmoud et al. (2019)

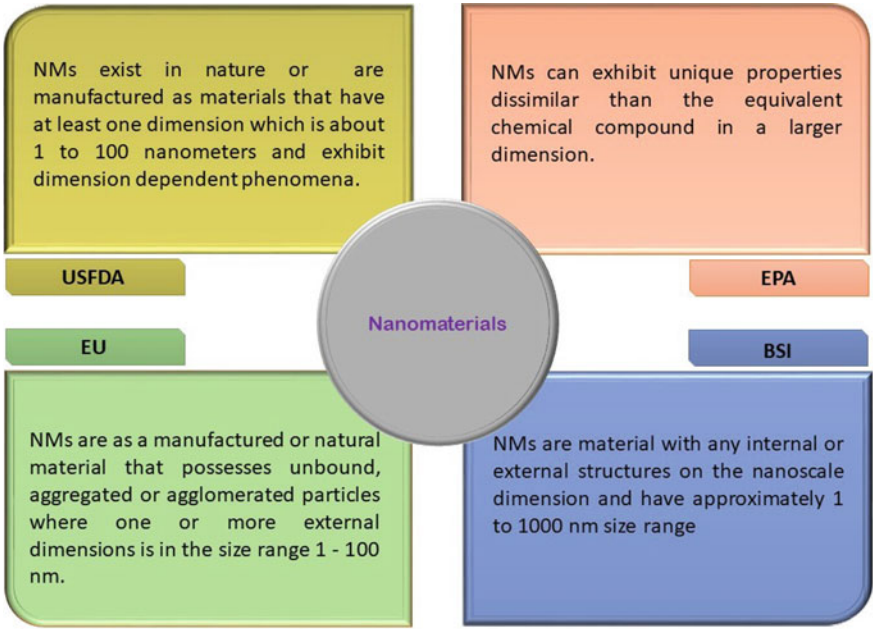

Fig. 16.1 Pictorial view of abiotic, environmental and nanoparticles mediated responses in plants

plants try different approaches for survival trough morpho-physiological adjustments for tolerence or avoidence (Bassett, 2013). However, under water stress, photosynthesis is limited with ultimate decline in plant biomass (Zarafshar et al. 2014). Pretreatment with silver nanoparticles helped in improvement of growth and biomass under water restricted environment (Khan et al. 2020). Silica nanoparticles enhanced plant overall weight and xylem water potential in water deficit *Crataegus sp* with elevated rate of photosynthesis and conduction of stomata (Ashkavand et al. 2015).

Foliar submission of different nanoparticles also enhanced the growth and productivity of water deficit plants. Treatment of selenium and silica nanoparticles preserved more photosynthetic pigments with enhanced water use efficiency (Zahedi et al. 2020). Delay in panical initiation time leading to improved grain yield in wheat was observed under zinc oxide nanoparticles application (Dimkpa et al. 2020).

Treatment with zinc oxide nanoparticles also stimulated macro- and micronutrients' uptake and elevated relative water content in eggplants under water deficit area (Semida et al. 2021). Copper nanoparticles improved the biomass and enhanced seed count and productivity of maize plants trending to reinforced drought stress tolerance (Nguyen et al. 2021). Titanium oxide nanoparticles elevated the carotenoids content and amended cell membrane damage and seed oil and protein (Aghdam et al. 2016). These studies indicated that more in-depth research is required for developing tolerance against drought with the help of nanoparticles.

16.3.2 Heavy Metal Stress

Metal uptake could be declined with application of silicon nanoparticles in rice (Cui et al. 2017) and minimized the growth inhibitory action of mercury in soybean (Li et al. 2020) and in wheat tissues (Jiang et al. 2021). Nanoparticles have different behaviour in different plants as zinc oxide nanoparticles and iron nanoparticles amplified the cadmium concentration in lettuce (Venkatachalam et al. 2017) while disclosure of plants to contrary form of nanoparticles boost the growth and declined the cadmium content in plants (Wang et al. 2012). Likewise, silicon nanoparticles mitigated the cromium morbidity in pea (Tripathi et al. 2015). Iron nanoparticles treatment increased the wheat growth and declined the metal phytoremediation (Konate et al. 2017). Zinc oxide nanoparticles elicited plant growth, and yield under cadmium stress in wheat (Rizwan et al. 2019a, b) Treatment with zinc oxide nanoparticles expedited less aggregation of arsenic in root and shoot (Yan et al. 2021). Copper nanoparticles has also critical role in reduction of cadmium transport in wheat (Noman et al. 2020).

16.3.3 Salinity

The major threat to modern agriculture is the salinity which inhibited and impaired the crop growth and development (Isayenkov 2012). Responses of plants to salinity could be ion-independent growth reduction (Rajendran et al. 2009) or formation of cytotoxic ion levels with slow metabolic methods (Roy et al. 2014).Various physio-molecular mechanisms help in development of tolerance to stress (Rajendran et al. 2009). Application of nanosilica decreased the hazardeous impact of salinity on germination, root length and plant dry weight in tomatos (Haghighi et al. 2012). Similarly; treatment of carbon nanoparticles stimulated the germination rate in lettuce (Baz et al. 2020).

Interaction of zinc oxide nanoparticles with salinity stress reversed the salinity induced antioxidants enzymes production in two cultivars of fenugreek (Noohpisheh et al. 2021). Zinc oxide nanoparticles also enhanced shoot and root elevation, plant weight, and leaf area in tomato (Faizan et al. 2021) with elevated chlorophyll and photosynthetic qualities, protein content and activities of antioxidative enzymes in salinity-stressed tomato plant. Priming with silver nanoparticles significantly increased the plant weight of salinity stressed wheat (Mohamed et al. 2017).

16.3.4 Miscellaneous

Increase or decrease on temperature (High temperature stress or low temperature stress or cold) also participate in the growth of plants. High temperature is a particular interest for major cash crops due to severe reduction in crop yield (Mondala et al. 2013) and numerous tactics such as hybridization (Zhao et al. 2008; Semenov and Halford 2009), genetic engineering (Barnabas et al. 2008), molecular markers-assisted selection and QTL mapping (Vinh and Paterson 2005) are in use to mitigate these hazardeous effects. High temperature significantly reduced the chlorophyll content and increased membrane injury index in wheat (Almeselmani et al. 2006). However, Treatment with silver nanoparticles protected wheat plants in contradiction of heat stress and improved length of root and shoot length with increase in root number and plant biomass (Iqbal et al. 2017). Titanium oxide nanoparticles built redox status of cold sensitive and tolerant chickpea plants (Mohammadi et al. 2013).

16.4 Conclusion and Prospects

Abiotic stresses and limited nutrient are main environmental conditions that decrease plant growth, productivity and quality (Gong et al. 2020). Abiotic stresses destructively impact the plant's growth and development trending to decline in productivity in terms of both the quality and quantity (Sharma et al. 2019). These abiotic stresses directly influence the plant by bringing changes in the structural and chemical composition of the plant. However, if plants are exposed or treated with nanoparticles then their response to stresses will be different. Nanoparticles have the ability to impact the hereditary material of the plant such as DNA and affect the growth of plants under various stresses. Nanotechnology has achieved enormous impulse in recent times because of the varietal utilization of nanoparticles in agriculture, drug and cosmetic industry. Their wide application raised concerns about their possible way out into the ecosystem and their interaction with the plants. Nanoparticles have the ability to alter the morpho-physiological responses of plants. Nanoparticles have also altered the expression of various genes and changed the chemical composition of various plants. This property could potentially be used to address various abiotic stresses. Various research groups are now focusing on this aspect of nanoparticles and drawing outstanding results. However, in future there is a need for more comprehensive studies, so that the effects of the practiced nanoparticles on plants can be determined well in time. There is also a desire to understand their impact on all kinds of living organisms in future because of their continuous entry into the environment with ultimate exposure of all kinds of lives on earth.

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Chapter 17
Plant Mediation to Tolerate Cadmium Stress with Selenium and Nano-Selenium

Ali Akbar Ghasemi-Soloklui, Fardad Didaran, Mojtaba Kordrostami, and Jameel M. Al-Khayri

Abstract After mercury and lead, cadmium is the third greatest hazard dangerous to the environment. Cadmium is non-essential biological functions metal element and has a series of harmful effects on the health of human, animal, and plants at low concentrations. Cadmium is unique heavy metal that causes health problems Plants in many areas are low or mildly polluted with cadmium may not display any toxicity problems. They can accumulate cadmium in their edible portions at levels that are higher than the permissible threshold for people. Plant foods are generally considered to be the most prevalent source of cadmium exposure in the population, and grains account for a considerable portion of total dietary intake. It is well documented that added at low concentrations of selenium exerts beneficial effects regulation of photosynthesis and respiration, increased antioxidant capacity, improvement of abiotic stress tolerance, and attracted attention in the alleviation of heavy metal toxicities stresses in different plant species. This chapter summarized how selenium and Nano-selenium can be mitigated cadmium stress in plants.

Keywords Heavy metal · Nanotechnology · Stress management · Toxicity

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17.1 Introduction

Cadmium is a soft, ductile, silvery white with blue tint, glossy, and electropositive element with atomic number 48, atomic mass 112, melting point 321 °C, and boiling point 765 °C, and it has no odor or flavor and is extremely dangerous. Cadmium is known to fourth most heavy metal toxic to plant (Qadir et al. 2004). It is non-essential biological functions metal element and has a series of harmful effects on the health of human, animal and plants at low concentrations (Solenkova et al. 2014). The ability of plants to uptake and cadmium accumulates capacity in different types of plants crop related to soil and environmental factors including; concentration of cadmium in soil, pH, temperature, redox potential, level of other mineral nutrition, soil organic matter and as well as Plant physiological properties such as species and cultivars' capacity to accumulate and translocate, sequestration inside root vacuoles, translocation in the xylem and phloem, and dilution within plant tissue (Ismael et al. 2019a). Selenium name comes from the Greek word for moon or Selene and was discovered by Jakob Berzelius in 1817. The selenium has been forming of the Group 16 (chalcogen element) with an atomic weight of 78.96 and Ionic radius of selenium similar chemical properties with sulfur (S). Selenium exists in both forms (inorganic and organic) in the environment (Gupta et al. 2017). The most common inorganic forms of selenium exist in five forms state to selenide (HSe^-), elemental selenium (Se^0), selenite ($HSeO_3^-$ and SeO_3^{2-}), and selenate (SeO_4^{2-}), whereas predominant organic forms of selenium in biological matter were methylated selenium compounds, selenoamino acids, and selenoproteins (Rizwan et al. 2021). Zhu et al. (2009) reported that both organic and inorganic forms of selenium can be uptake by plants. Under Cadmium stress, the heavy metal Cadmium is easily transported from soil to plants (Riaz et al. 2021a, b). When considering the harmful effects of heavy metals on plants, it's important to remember that toxicity is determined by the external bioavailable metal content, the exposure period, the plant genotype, and the plant's overall state. Furthermore, dose–response curves for essential and non-essential components differ (Fig. 17.1). Inorganic Selenium was found to reduce Cadmium absorption in tomato, pakchoi, wheat, and other plants in studies (Wu et al. 2013; Zhou et al. 2020). Also, the addition of Nano-Selenium, Selenium (IV), and SeleniumMet decreased Cadmium in rice tissues (shoots and roots), with the SeleniumMet treatment having the greatest impact. Low levels of Selenium increased the proportion of coarse roots, resulting in lower Cadmium absorption; however, at higher Selenium and Cadmium concentrations, Selenium impeded plant development rather than reducing Cadmium toxicity, which was attributed to increased root cell permeability to Cadmium owing to root cell membrane degradation.

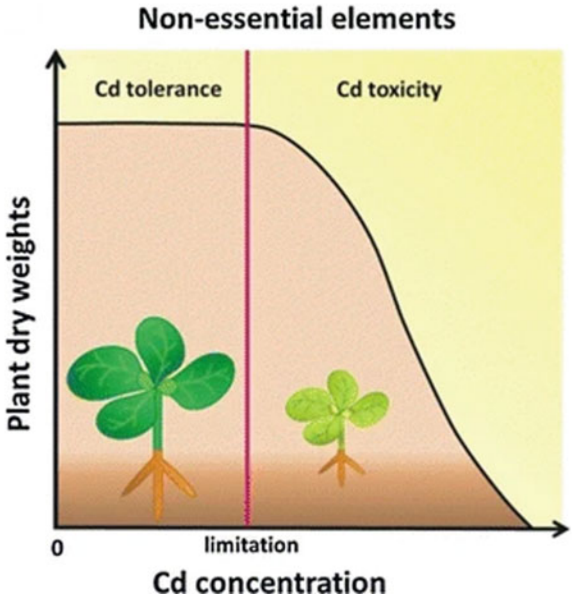

Fig. 17.1 Plant dose–response curves for essential and non-essential micronutrients

17.1.1 *Cadmium and Health Effects*

The base of origin product can be divided into two types of cadmium; one is from natural sources, the other type is of anthropogenic origin, that can be widely present in soil, water, and the atmosphere (Alloway 2012; Shahid et al. 2016; Wang et al. 2015a, b). In nature, cadmium is released into the environment immobilized by interacting with iron (Fe) and manganese (Mn) oxides and can also be atmospherically deposited on rain, dust, snow and mainly from weathering of soil parent material and volcanic activities (Cook et al. 1995; Hayat et al. 2019; Zhang et al. 2013). Anthropogenic cadmium emissions, on the other hand, are much more serious, accounting for 8000 to 10,000 mt per year. Industrial activity (manufacturing of plastics, chemical stabilizers, paint pigments processes, metallic coatings and alloys), chemical fertilizers, contaminated sewage sludge and waste water, sewage effluents, and agricultural run-offs are the major targets of anthropogenic activities (IPCS 1992; Ji et al. 2011; Misra et al. 2005; Xue et al. 2014; Zarcinas et al. 2004). Cadmium concentrations in uncontaminated soils are typically around 0.5 mg kg^{-1} (Vahter et al. 1991), but because it is readily absorbed by plants growing on cadmium-supplemented soils, cadmium concentration in polluted soils can be 10–500 times greater than in non-polluted soils and may directly contributing to plant and human health (Yanqun et al. 2005). Cadmium contamination in food is mostly caused by widespread low-level soil pollution and effective cadmium soil-to-plant transfer. And since cadmium cannot be destroyed and has a biological half-life of up to 30 years, it is possible that it may accumulate in the body through the food chain and finally end up in the human kidney (Ismael et al. 2019a). After mercury and lead, cadmium is the third greatest hazard dangerous to the environment, according to the US Environmental Protection Agency (EPA) (Jamers et al. 2013). Also, cadmium is the unique heavy metal that causes health problems, plants in many areas that are proven low or mildly polluted with cadmium may not display any toxicity problems, but they can accumulate cadmium in their edible portions at levels that are higher than the permissible threshold for people. When these plants reach the food chain, they become extremely toxic and can cause a variety of health risks (Ismael et al. 2019a). The authors demonstrate that 98 percent of cadmium intake is expected to come from terrestrial foods, 1% from aquatic foods, and 1% from cadmium in drinking water (Van Assche 1998). Thus, plant foods are generally considered to be the most prevalent source of cadmium exposure in the population, and grains account for a considerable portion of total dietary intake (Huang et al. 2020). The FAO/WHO Codex Alimentarius Commission is debating a limit of 0.1 mg kg^{-1} for cereal grains and oilseeds traded on global markets while the World Health Organization determined a maximum provisional acceptable intake limit of 60–70 g cadmium per day for adults (WHO 1973). Thus, even at low-level chronic exposure for both people and animals at plant tissue amounts that are naturally not phytotoxic, extremely substantial health problems might occur.

17.1.2 Selenium in Soil and Plants

The average selenium concentration in different soils is 0.4 mg/Kg in worldwide; however mountainous countries such as Finland, Sweden, and Scotland are generally deficient selenium value in soil whereas dried areas in world are selenium rich regions (Gupta et al. 2017). Generally, the bioavailability of selenium in the soils depends on its chemical nature of selenium form and is affected by pH, local precipitation, application of mineral fertilizers, redox potential, organic matter, competitive ions, microbiological activity of soil, plant species and cultivars (Hartikainen 2005; Hawrylak-Nowak et al. 2015; Zhang et al. 2007). Sieprawska et al. (2015a) suggest that the concentration of selenium in the soil determines accumulation of this nutrition element in different parts of plants. Based capacity of accumulation selenium inside their cells, Galeas et al. (2007) were classified crop plants into three groups including; non-accumulators groups which usually contain less than 25 μg Se/g dry weight (DW); indicators groups accumulating from 25 to 1000 μg Se/g DW and hyper accumulators groups having the ability to absorb higher amounts of their cells more than 1000 g in 1 g of DW. Selenium is the most important element for animals, humans and plants, although there is no evidence of selenium as considered an essential element for higher plants. However, several previous reports showed that selenium exerts beneficial effects on important to plays vitals roles in plants and promote plant growth (Jia et al. 2018; Kamran et al. 2020; Pilon-Smits et al. 2017). It is well documented that added at low concentrations of selenium exerts beneficial effects regulation of photosynthesis and respiration, increased antioxidant capacity, improvement of abiotic stress tolerance, and attracted attention in the alleviation of heavy metal toxicities stresses in different plant species (Dai et al. 2019; Duan et al. 2019; Ulhassan et al. 2019; Wang et al. 2015a, b). Moreover, this devastating selenium could affect crop plant quantity and quality in several plants. Thus, selenium is a considerably beneficial element for physiological and biochemical responses in different plant species.

17.1.3 Se Interactions with Cadmium in Plants

Due to the unfavorable impact on physiological processes such as photosynthesis, food absorption, and water balance, plants growing in excess Cadmium display stunted growth and development. Selenium can have a significant effect on tolerance to this heavy metal (Hasanuzzaman et al. 2020; Seifikalhor et al. 2020; Wu et al. 2013). Selenium's possible function in reducing heavy metal (particularly Cadmium) toxicity in plants has received more attention in recent years. Wan et al. found that rice seedlings fed with selenite [Selenium (IV)] and selenate [Selenium (VI)] had lower root-to-shoot Cadmium translocation. In plants, Selenium may interact with Cadmium to produce a stable Selenium-Cadmium complex (Ryant et al. 2020; Xia et al. 2020; Zohra et al. 2021; Zwolak 2020). In *Brassica juncea* L., Ahmad et al. discovered that applying Selenium (IV) reduced lipid peroxidation and Cadmium absorption, transport, and distribution while minimizing the Cadmium oxidant impact (Hossain et al. 2021; Zohra et al. 2021). Lin et al. also discovered that Selenium (IV) can limit the availability of Cadmium. Under Cadmium stress, Selenium aids in the repair and regeneration of the cell's structure (cell membrane and chloroplast) (Lin et al. 2012). Selenium (IV) addition decreased Cadmium buildup and relieving Cadmium toxicity in pepper, according to Feng et al., via increasing chlorophyll concentrations and overall antioxidant activity (Feng et al. 2021). These findings show that Selenium, particularly Selenium (IV) and Selenium (VI), has positive effects in plants under Cadmium stress. In the environment, there are several types of Selenium. Under a well-drained mineral environment, Selenium (IV) is the major form of Selenium; but, in alkaline and well-oxidized circumstances, Selenium (VI) is the dominating species (Medrano-Macías et al. 2018; Riaz et al. 2021a, b; Rizwan et al. 2020). Organic forms of Selenium account for a significant portion of the Selenium in soil. Because organic Selenium is quickly absorbed by plant roots, even low amounts of organic Selenium in the soil are significant (Ryant et al. 2020; White 2018). According to Schiavon et al., wheat and canola absorb selenomethionine (SeleniumMet) 20 times faster than Selenium (VI) or Selenium (VII) (IV) (Malagoli et al. 2015). Nano-selenium, which is employed in agriculture, medicinal therapy, and Selenium fertilization, has received a lot of interest in recent years (Chakraborty et al. 2021; El-Ramady et al. 2015, 2020). The reduction of Selenium oxyanions results in Nano-sized elemental Selenium (selenium nanoparticles, Selenium NPs). Nano-selenium, which is employed in agricultural areas, medicinal therapy, and Selenium fertilization, has gotten a lot of interest in recent years (Medrano-Macías et al. 2018; Zohra et al. 2021). In the environment, Nano-sized elemental Selenium (selenium nanoparticles, Selenium NPs) generated by the reduction of Selenium oxyanions via biotic or abiotic routes are abundant. In the roots and shoots, plants absorb Nano-selenium and convert it to inorganic Selenium molecules like Selenium (IV) and Selenium (VI). At the whole-plant level, Selenium (IV) and Selenium (VI) significantly decrease Cadmium absorption; nevertheless, the mechanisms that ameliorate Cadmium stress remain unexplored (Hasanuzzaman et al. 2020; Ismael et al. 2019a; Rizwan et al. 2020; White 2018). Furthermore, the Selenium species implicated in successful Cadmium phytotoxicity mitigation are unclear.

The addition of selenium (VI) did not cause a significant change in root cadmium level, but it did cause a decrease in shoot Cadmium content. Yu et al. also found that Selenium (IV) decreased Cadmium levels in Pakchoi shoots whereas Selenium (VI) enhanced it. Both Selenium (IV) and Selenium (VI) decreased Cadmium absorption in tomato, according to Alves et al. (*Solanum lycopersicum* L.) (Adnan 2020; El-Ramady et al. 2015; Hasanuzzaman et al. 2020; Ismael et al. 2019a; Riaz et al. 2021a, b). These findings indicate that the particular impact is dependent on Cadmium dose, plant species, and Selenium species. The positive impact of selenium was strongly connected to the reduction in Cadmium absorption or translocation towards the shoots, whereas roots function as a barrier against Cadmium translocation (Xia et al. 2020; Yin et al. 2019; Zhou et al. 2020; Zohra et al. 2021; Zwolak 2020).

Cd exposure causes the buildup of thiol compounds in living organisms, according to several lines of evidence. Thiol sulphydryl groups (e.g. GSH) may bind to HMs like Cd, resulting in effective metal sequestration. Previous research has shown demonstrated that intolerant plants' activity of γ-glutamyl-cysteine synthase, a rate-limiting enzyme in GSH metabolism, is lower than that of sensitive plants (Zagorchev et al. 2013). Cd sensitivity was also seen in transgenic Arabidopsis plants with bacterial γ-glutamyl-cysteine synthetase (Xiang et al. 2001). These data lead us to believe that different plants' Cd tolerance is based on distinct processes. Abiotic stresses such as Cd shock may cause GSH to "transiently" rise. Plants, on the other hand, use a variety of ways to commit high Cd levels. The system that prevents Cd absorption or translocation in plants is one example. Cd penetrates plants primarily through root epidermal cells at the cellular level. This entry is associated with three well-known steps: I Cd exchange with $H_2CO_3{}^-$released H^+, (ii) recruitment of Fe^{2+}, Zn^{2+} (belonging to ZIP family transporters) such as Yellow Stripe 1-Lik (YSL) protein and Ca^{2+} channels, and (iii) formation of metal–ligand complexes with mugineic acids (MA) in soil, and (iv) authorization for entrance (Seifikalhor et al. 2020) (Fig. 17.2).

17.1.4 Selenium Roles to Mitigate Cadmium Toxicity

Selenium reduces Cadmium's buildup and relieves the symptoms of this harmful heavy metal poisoning and plant growth inhibition. Plants have a variety of processes that might be involved in heavy metal detoxification (Adnan 2020; Lin et al. 2012). These systems appear to be engaged mainly in preventing toxic concentrations from building up at sensitive areas inside roots, reducing harmful consequences. The

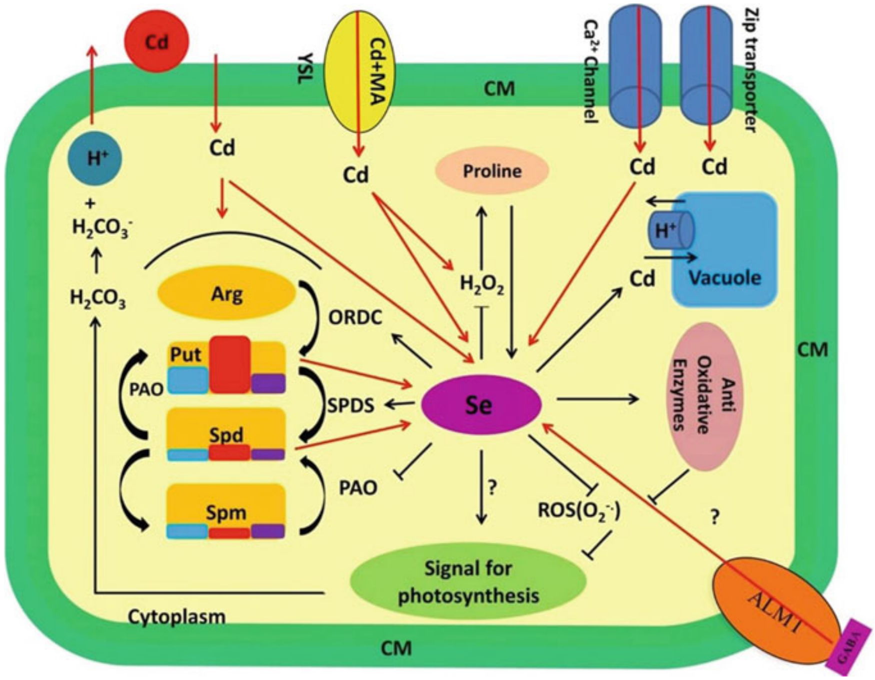

Fig. 17.2 Se's regulating role in plant cells under Cd stress is depicted schematically. Se is engaged in a variety of metabolic and molecular activities (black arrows and lines), and the cell's Se content is affected by a variety of routes (red arrows)

hydroponic experiment revealed that most of the Selenium absorbed by plants was stored in the roots (White 2018; Wu et al. 2013; Zhou et al. 2020; Zohra et al. 2021; Zwolak 2020). According to several studies, Selenium is inadequately translocated to shoots in selenite-treated plants.

Meanwhile, compared to Cadmium-alone treatment, all plants grown on Cadmium + Selenium media revealed a typical response pattern that reduced Cadmium levels in both leaves and roots (Rizwan et al. 2020; Ryant et al. 2020; Seifikalhor et al. 2020). Cadmium fluorescence location and intensity show that Selenium significantly reduced Cadmium accumulation in the roots of Cadmium-treated rice plants. Different studies show that Hg- and Selenium-containing protein complex forms and is present in plant roots. This compound is sturdy enough to prevent mercury from reaching the plant's aerial components (Adnan 2020; El-Ramady et al. 2020). Selenium ions are thought to be co-transported with Cadmium ions by the same protein carriers, resulting in a reduction in Cadmium at metabolically active membrane regions. Both Cadmium and Selenium are attached to thiol groups of cysteine, an amino acid found in specific proteins, according to Filek et al. (2008). As a result, competition for specific protein binding sites may explain some of the lower Cadmium absorption and Selenium's protective impact against Cadmium toxicity.

The addition of 3 μM Selenium to 50 μM Cadmium resulted in a significant increase in the Cadmium-induced reduction in plant growth, as measured by the SPAD value, plant height, root length, and biomass (Filek et al. 2008; Hasanuzzaman et al. 2020; White 2018). According to Pennanen et al. (2002) Selenium promotes plant development via increasing starch accumulation. In the chloroplast, Selenium is assimilated, which needs reducing power from NADPH and GSH. This can change the redox status of chloroplasts, impacting biomass production (Sieprawska et al. 2015a, b). The current findings show that Selenium may effectively relieve Cadmium-induced growth inhibition as well as the symptoms of chlorosis and necrosis on leaves, suggesting that low micromoles of Selenium might have positive effects on plants, even though Selenium is poisonous at significant concentrations (Chakraborty et al. 2021; Feng et al. 2021; Hossain et al. 2021; Riaz et al. 2021a, b; Zhou et al. 2020; Zohra et al. 2021; Zwolak 2020).

Selenium has also been used as a soil additive to decrease Cadmium toxicity in various plant species at various Cadmium concentrations. When Selenium was administered to plants growing under Cadmium stress, the Cadmium content in Chinese cabbage (*Brassica rapa*) and lettuce (*Lactuca sativa* L.) reduced dramatically, but several good mineral elements were concentrated, such as Mn and Mg rose to some amount (Hu et al. 2014; Sillanpää et al. 1992). The soil application of Selenium to *Brassica juncea* reversed the unfavorable effects of Cadmium and increased plant growth, relative water content (RWC), pigment content, and protein content (Pedrero et al. 2008). Selenium may also reduce the buildup of H_2O_2 and lipid peroxidation, boost the activity of antioxidant enzymes like CAT and APX, and regulate Cadmium accumulation in roots and shoots. When Selenium was applied to wheat under Cadmium stress, it reduced ethylene levels and increased proline accumulation and the activities of glutathione reductase (GR) and glutathione peroxidase (GPX), alleviating Cadmium-induced oxidative stress. Selenium substantially decreased Cadmium accumulation in Cadmium-treated pepper plants and enhanced fruit number, fruit diameter, and fruit output per plant in Cadmium-treated plants. When Selenium was given to the Cadmium-treated plants, the pepper fruits' chlorophyll contents and total antioxidant activity increased (Khan et al. 2015; Sun et al. 2013).

Abiotic and biotic stressors both include oxidative stress. A major cell imbalance between ROS generation, such as $O^{2\bullet}$, $^{\bullet}OH$, and H_2O_2, and antioxidative enzymes causes this process, leading to catastrophic physiological problems. Higher lipid peroxidation in plant cells might cause Cadmium harm, as seen by increased MDA levels. Several studies have shown that Selenium reduces oxidative stress caused by Cadmium, as seen by lower levels of $O_2^{\bullet}$, H_2O_2, and MDA (Qi et al. 2021; Wu et al. 2020). ROS can cause peroxidation and degradation of the lipid bilayer of the cell membrane, which has an impact on cell function (El-Ramady et al. 2015; Hossain et al. 2021; White 2018). Plant cell membranes are one of the initial targets for various stressors and maintaining membrane integrity and stability is critical for stress tolerance. The cell viability results in roots match those of Cadmium stress-induced ROS measurements. It is conceivable that a fraction of the oxidative radicals is eliminated non-enzymatically in the presence of Selenium (Tang et al. 2015).

Selenium has been found to alter the activity of oxidoreductase enzymes in wheat, potato, and soybean. Selenium exerts synergistic effects on the transcription of ant oxidative enzymes including CuZnSOD and GPX in plants, according to Seppänen et al. 2004 Selenium enhances H_2O_2 scavenging by raising the activity of GPX, which was first discovered as an abiotic stress-responsive enzyme. However, additional research is needed to identify the role of Selenium in rice's antioxidative system in the case of Cadmium poisoning (Kantola et al. 2004; Mabeyo et al. 2015; Malagoli et al. 2015; Sieprawska et al. 2015a, b; Tang et al. 2015).

17.1.5 Mechanisms of Selenium Strategies Towards in Cadmium Toxicity

In a variety of methods, Selenium can prevent the oxidative damage induced by Cadmium. Selenium lowered Cadmium concentrations in the leaves, roots, and stems of diverse plants cultivated in a Cadmium-contaminated hydroponic culture, restored root cell viability, and significantly reduced O_2, H_2O_2, and malondialdehyde accumulation in various plant tissues. It also increased the root H^+- and Ca^{2+}-ATPase activity in Cadmium-stressed roots (Lin et al. 2012; Medrano-Macías et al. 2018; Seifikalhor et al. 2020). Furthermore, Selenium supplementation of Cadmium-treated plants decreased PC accumulation in roots but did not affect PC concentration in cucumber leaves. Selenium treatments, either alone or in combination with Si, could stimulate the efficiency of the GSH–AsA cycle by increasing glutathione (GSH) and ascorbate (AsA) concentrations in Chinese cabbage tissues under Cadmium stress, in addition to increasing the activities of antioxidant enzymes such as SOD, CAT, and APX (Štajn et al. 1997; Wu et al. 2017). Selenium has been shown to boost GSH–PC levels in various plants. When exposed to heavy metals, Selenium may protect against cadmium toxicity by raising the concentration of GSH–PCs, implying that Selenium may protect against cadmium toxicity by increasing the concentration of GSH–PCs. In the case of rapeseed seedlings, similar conclusions were also found (Li et al. 2016). In an in vitro experiment, chloroplasts from rape leaves were cultivated and given 2.0 mM Na_2SeO_4 and/or 400 mM $CadmiumCl_2$, and selenium was shown to partially counteract the harmful effects of cadmium, resulting in an increase in chloroplast size and rebuilding of the chloroplast ultrastructure (Das et al. 2018; Van Puymbroeck et al. 1982).

Generally, the effect of Selenium on cadmium uptake was discovered to be dependent on the degree of cadmium contamination. Thus, at low cadmium concentrations, Selenium might inhibit Cadmium absorption in plants and increase biomass; but, at high cadmium concentrations, the addition of selenium enhanced cadmium uptake and negatively impacted plant development, particularly at high Selenium concentrations. Nonetheless, selenium improved the absorption of other nutrients, including Ca, Mg, Mn, Cu, and Zn in both circumstances, but cadmium alone inhibited their uptake (El-Ramady et al. 2015; Malagoli et al. 2015; Medrano-Macías et al. 2018;

Ryant et al. 2020). In a study comparing the effects of various forms of Selenium on cadmium uptake in plants over time, selenite boosted cadmium uptake after short-term exposure, but selenate did not affect cadmium flow, ruling out any potential rivalry between selenium and cadmium on the root surface. Indeed, the root cells take up cadmium and selenate (selenite) through ZIP and sulfate (phosphate) transporters, respectively, indicating that they do not use the same transporters (Ismael et al. 2019b). However, when the culture time was extended, selenium appeared to diminish cadmium absorption by roots and root-to-shoot translocation, which was more noticeable at higher Selenium concentrations. As a result, selenium inhibits cadmium absorption and translocation to higher plant tissues and the toxic symptoms associated with it. Selenium has multiple hypothesized methods to decrease cadmium-induced oxidative damage due to its diverse positive activities in plants. Although certain postulated functions are supported by evidence in most publications, other possibilities cannot be ruled out (Gupta et al. 2016; Khan et al. 2015; Li et al. 2016; Mabeyo et al. 2015; Malagoli et al. 2015; Sieprawska et al. 2015a, b; Tang et al. 2015).

Aside from the chemical functions that selenium may play in cadmium absorption, translocation, and sequestration, selenium has a significant capacity to interact with heavy metals including cadmium, Hg, Ag, and Tl to form complexes that are not accessible for uptake. When selenium is given to soil or hydroponic culture that has been polluted with cadmium, one probable mechanism is that it binds the Cadmium and converts it into other forms that are not available for absorption, reducing the amount of Cadmium in plant tissues. Short-term exposure to selenite or selenate (60 min) modestly increased Cadmium inflow in roots and accelerated Cadmium uptake, suggesting that Selenium and Cadmium do not compete for absorption on the root surface. The inclusion of Selenium in the culture solution, on the other hand, lowers Cadmium absorption when the culture duration is increased (Adnan 2020; Ali et al. 2020; Yin et al. 2019).

In general, there is little research on the effect of Selenium on Cadmium toxicity in plants at the molecular level, including how it changes the expression levels of genes involved in Cadmium absorption and processing. However, a few reports may be expanded upon to help us better comprehend this critical point. When plants suspension cells were exposed to Cadmium in the presence or absence of Selenium, the Selenium-pretreated plant cells had lower expression levels of the OsLCT1, OsNramp5, OsNramp1, OsIRT1, and OsIRT2 genes, as well as decreased Cadmium absorption. These data suggest that Selenium inhibits the expression of OsLCT1, OsNramp5, OsNramp1, OsIRT1, and OsIRT2 in cells, lowering Cadmium absorption. However, when the Cadmium content in the medium rose, the expression level of OsHMA3 increased dramatically in the Selenium-pretreated cells, but no noticeable changes in OsHMA3 expression were seen in the cells lacking Selenium (Cui et al. 2018; Wang et al. 2020). This finding suggests that adding Selenium to plant cells increased OsHMA3 overexpression, which improved their tolerance to toxic cadmium (Cui et al. 2018).

Cadmium is mainly linked to S-containing ligands such MTs, GSH, and PCs, which are involved in heavy metal sequestration and detoxification. Selenium can trigger GSH–PCs biosynthesis in a variety of plants that have been exposed to heavy metals (Rana et al. 1996). This suggests that Selenium may protect against cadmium toxicity by raising the concentration of GSH–PCs. Selenium also increased plant tolerance to Cadmium by upregulating PCS1 expression in *B. napus* leaves under cadmium stress. Even though the cadmium concentration in the Selenium-treated plants was more significant than in the non-treated plants, this cadmium tolerance improved plant development. Selenium also increased IRT1 expression in root tissues, resulting in increased Cadmium absorption by the roots, which was in line with the elevated cadmium level in the roots of Selenium-treated plants compared to cadmium-only plants(Ismael et al. 2018). Furthermore, Selenium treatment increased cadmium root-to-shoot translocation by upregulating the HMA2 and HMA3 genes.

17.1.6 Optimal Dosages of Selenium Reduce the Levels of ROS

The current method for applying Selenium fertilizer as a foliar spray or a base fertilizer has been utilized to boost Selenium content in the edible section of crops while also counteracting the damage caused by various environmental conditions. For instance, Waisberg et al. (2004) discovered that adding selenite to lettuce (*Lactuca sativa* L.) plants exposed to Pb and Cadmium reduced the accumulation of these heavy metals while increasing the absorption of some critical elements, including Selenium, in a field experiment. However, while employing this technique, the question of what is the ideal Selenium dose frequently arises (Waisberg et al. 2004). Selenium has a two-fold influence on plant growth. It may boost plant development and neutralize many forms of environmental stressors, including HM, at low concentrations, but it can also function as a pro-oxidant and harm plants in excessive quantities. In ryegrass (*Lolium perenne*), 1 mg/ kg Selenium (H_2SeO_4) injected into the soil was thought to be mildly hazardous (Luo et al. 2011).Selenium can affect the formation and quenching of ROS either directly or indirectly through antioxidant regulation. The modulation of ROS levels by Selenium might be an important strategy for plants to combat environmental stress. Under normal circumstances, ROS generation in plant cells is kept to a minimum, i.e. less than 240 M s^{-1} $O_2^{\bullet}$ and 0.5 M H_2O_2 in chloroplasts. In plants exposed to a variety of environmental challenges, a modest addition of Selenium to the growth substrates can minimize excess ROS production, particularly $O_2^{\bullet}$ and/or H_2O_2(El-Ramady et al. 2015; Filek et al. 2008; Kantola et al. 2004; Sun et al. 2013; White 2018). Selenite relieved Al-induced oxidative stress in ryegrass roots, according to Cartes et al. (2010), mostly via increasing the spontaneous dismutation of $O_2^{\bullet}$ to H_2O

$_2$(Cartes et al. 2011). Mroczek-Zdyrska and Wójcik (2012) discovered that a modest dose of Selenium (1.5 M Na_2SeO_3) reduced $O_2^{\bullet}$ levels in Pb-exposed *Vicia faba* L. roots(Mroczek-Zdyrska et al. 2012). The addition of 2 M Selenium (Na_2SeO_4) to rape (*Brassica napus*) seedlings subjected to 400 or 600 M Cadmium was shown to reduce H_2O_2 buildup in the plant's shoots and roots in one experiment (Sieprawska et al. 2015b). Reduced ROS levels in plants exposed to other stresses, such as grain sorghum (*Sorghum bicolor* (L.) seedlings exposed to high temperature (O_2 and H_2O_2) (Na_2SeO_4), wheat (*Triticum aestivum* L., cv) seedlings exposed to UV-B radiation stress ($O_2^{\bullet}$) (Na_2SeO_3) and cold stress ($O_2^{\bullet}$) (Na_2SeO (H_2O_2).The spontaneous dismutation of $O_2^{\bullet}$ into H_2O_2 (without catalysis by the SOD enzyme), the direct quenching of $O_2^{\bullet}$ and OH by Selenium compounds, and the control of antioxidative enzymes have all been postulated as probable reasons for the drop in $O_2^{\bullet}$ levels when suitable amounts of Selenium were administered.

17.1.7 Conclusion and Prospects

Cadmium is a common contamination that is very hazardous to the environment, making it the third most dangerous contaminant (after mercury and lead). Cadmium has negative health effects on humans and is the only metal that causes health hazards to both people and animals at non-phytotoxic plant tissue quantities. Uptake by roots, loading to xylem, translocation to shoots, and lastly phloem transfer to seeds are the four primary phases for cadmium, like other heavy metals, to reach upper plant parts such as leaves and fruits. Cadmium (re)distribution between roots and shoots is a dynamic process mediated by metal transporters at the plasma membrane of the root, xylem loading/unloading, and phloem loading/unloading. These mechanisms involve many channels and metal transporters, which require a thorough understanding to manipulate cadmium translocation/accumulation in various plant tissues, including seeds, which are critical in the food chain. Cadmium stress inhibits plant development through various causes, including reduced water and nutrient intake, photosynthesis, carbon and nitrogen assimilation, oxidative damage, and so on. Essential plant nutrients have been shown to reduce Cadmium toxicity in plants and regulate the accumulation of cadmium and other heavy metals in their edible sections. Many mechanisms for reducing cadmium toxicity in plants using trace levels of Selenium have been proposed, including (i) regulating reactive oxygen species and enzymatic and non-enzymatic activities; (ii) inhibiting Cadmium uptake and translocation and manipulating its fractions in plant cells; (iii) rebuilding damaged cell membranes, chloroplast structures, and components of the photosynthetic system damaged by Cadmium; and (iv) regulating essential element uptake. Selenium can change the expression level of various genes involved in the absorption, translocation, and detoxification of Cadmium at the molecular level, albeit this has received little attention and requires further research.

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Chapter 18
Synthesis and Applications of Cellulose Nanomaterials Derived from Agricultural Waste and Byproducts

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Abstract Cellulose is widely used as a renewable natural polymer for the preparation of various kinds of nanomaterials. Aside from being a nanostructured material, it has also other characteristics in producing different kinds of nanomaterials. Aside from bacterial nano cellulose, cellulose is also a bio-available source of cellulose.

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This chapter discusses its various structural characteristics to analyze the main structural features of nano cellulose. It shows that these materials have attractive and effective characteristics. The lateral portion of nano crystallites of cellulose is the same as that of elementary nanofibrils, though its length can vary from 50 to 200 nm. Heterogeneous acid hydrolysis is a process that involves the use of enzymes to increase the cellulose's micro and nanoparticles' crystallinity. It can also produce higher-quality aggregates. This chapter also covers various experimental procedures for the production of cellulose nanomaterials and the uses of nano-cellulose.

Keywords Cellulose nanocrystals · Characterization · Nanocellulose nanofibrils application

18.1 Introduction

Cellulose is the primary component of the plant cell wall. It can be extracted from various sources, such as wood, algae, fungi and bacteria. The plant cell wall also contains hemicellulose, lignin and small amounts of extractives (Chen et al. 2015). Due to their energy-efficient nature, agricultural byproducts are more advantageous in terms of processing than their counterparts. Although cellulose has been used in high-value-added applications, its hygroscopic nature makes it hard to perform these tasks and is suitable for many high-end processes (Zhang et al. 2022).

Due to its renewable nature and its various advantages, nano cellulose has gained increasing attention in the fields of biomedical engineering and materials science. Its various applications include biocompatibility, flexible electronic components and medical devices. Although various methods have been proposed for the production of nano cellulose, the commercial process still involves harsh chemicals. This is the reason why the process should be developed as a sustainable and environment-friendly one (Cindradewi et al. 2021; Hofmann and Reid 1929).

Numerous studies have been published in the field of nano cellulose (Das et al. 2022a, b; Hoenders et al. 2018; Oprea and Panaitescu 2020; Zeng et al. 2020). These studies mainly focused on the various aspects of nano cellulose production and its applications. It is usually necessary to carry out a detailed study to analyze the various steps involved in the cellulose extraction process. Due to the increasing importance of green and sustainable materials in the production of various high-value products, this field has gained widespread acceptance (Nehra and Chauhan 2021). This field of research has garnered the interest of various individuals and industries due to the

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potential of these materials to be used as an alternative energy source and a way to reduce greenhouse gas emissions.

Among the promising candidates for the abundant availability of resources are starch, cellulose, chitin, gelatin and chitosan. Among these, cellulose is the most abundant renewable compound. Cellulose is a fascinating polymer that has the potential to be used in various industrial applications. Its inexhaustive nature makes it an ideal source of raw materials (Siqueira et al. 2019). The properties of cellulose can be further enhanced by bundling them together, which can generate highly ordered regions. These materials are then considered nano-particles. These materials are abundant and renewable and they have various characteristics such as low thermal conductivity, high strength and low density (Ho et al. 2020).

Usually, nano cellulose can be divided into two main classes: nanostructured materials and nanofibers. The former can be made up of cellulose microcrystals and nanofibrils, while the latter can be derived from bacterial cellulose. The characteristics of nano cellulose vary depending on its origin and processing conditions (Yu and Yan 2017). For instance, the size and morphology of nano cellulose are related to the origin and processing conditions. The advantages of nano cellulose in terms of its physical and chemical properties, as well as its 3D hierarchical structure, have numerous applications in various industries (Siqueira et al. 2019; Yu et al. 2020). The increasing demand for nano cellulose has led to the creation of new jobs in the field of nano cellulose.

With its numerous industrial applications, nano cellulose has the potential to transform many industries. Its versatile properties can be utilized in various fields such as biomedical products, food coatings and various other industries (Yu et al. 2020). The search for novel applications and improving properties of nano cellulose-based products are driving forces for R&D in various research groups. Several literature reviews have been published in the last few years that mainly focused on the production and their modification (Yu and Yan 2017; Siqueira et al. 2019; Zhang et al. 2020; Zheng et al. 2020). This chapter presents an overview of cellulose including its structure and source, nomenclature and types, methods of preparation, characterization, properties and applications.

18.2 Source of Cellulose

Agricultural residues, water plants, grasses, and other plant substances are all sources of cellulose. They contain hemicelluloses, lignins, and tiny amounts of extractives in addition to cellulose (Hon 1996; Seddiqi et al. 2021) (Table 18.1). Commercial cellulose manufacturing focuses on either harvested sources like wood or naturally pure supplies like cotton. Several bacteria from the genera Acetobacter, Agrobacterium, Sarcina and Rhizobium produce cellulose, which is becoming increasingly important (Jonas and Farah 1998; Tarchevsky and Marchenko 1991). Bacterial cellulose (BC) is often very pure (no lignin or hemicelluloses), very crystalline and has a high degree of polymerization (DP).

Table 18.1 Degree of polymerization of cellulose from various sources

Source	Type	Degree of polymerization	References
Algae		2500–4300	Guo et al. (2017), Hallac and Ragauskas (2011)
Bacteria		7000–16,000	Hallac and Ragauskas (2011), Tahara et al. (1997)
Wood	Wood from various species	6000–10,000	Hallac and Ragauskas (2011)
	Wood pulp	2000–4000	Henriksson et al. (2007), Sehaqui et al. (2011), Shimizu et al. (2016), Sjöström and Westermark (1999)
	Wood Cellulose nanofibers (CNF)	250–3500	Benítez and Walther (2017), Guo et al. (2017), Henriksson et al. (2007, 2008), Kurihara and Isogai (2015), Shinoda et al. (2012)
Plants	Cotton	10,000–15,000	Hallac and Ragauskas (2011), Kumar et al. (2009)
	Corn	1700	Xu et al. (2009)
	Wheat straw	2600	Jahan and Mun (2009)
	Jute	1900	Jahan and Mun (2009)
	Bagasse	1000	Hallac and Ragauskas (2011)
	Corn stover	2500	Hallac and Ragauskas (2011)
	Corn kernel	1700	Hallac and Ragauskas (2011)
Tunicate		700–3500	Šturcová et al. (2005), Zhao and Li (2014), Zhao et al. (2015)
Wood	Wood from various species	6000–10,000	Hallac and Ragauskas (2011)
	Wood pulp	2000–4000	Henriksson et al. (2007), Sehaqui et al. (2011), Shimizu et al. (2016), Sjöström and Westermark (1999)

(continued)

Table 18.1 (continued)

Source	Type	Degree of polymerization	References
	Wood Cellulose nanofibers (CNF)	250–3500	Benítez and Walther (2017), Guo et al. (2017), Henriksson et al. (2007, 2008), Kurihara and Isogai (2015), Shinoda et al. (2012)

Source Seddiqi et al. (2021)

Algae (*Valonia ventricosa*, *Chaetamorpha melagonicum*) are other sources of high-crystallinity cellulose that have been used to investigate the polymorphs of the biopolymer. Valonia cellulose can also be present in fungal cell walls. There are also various animal-derived celluloses, one of which, Tunican, a cell wall component of ascidians, has been extensively researched.

18.2.1 Cellulose Derived from Traditional Sources

18.2.1.1 *Wood*

The production of wood via the biogenesis of cell walls proceeds in aqueous environments, while the cell wall's main component, cellulose, has insoluble in water. A hierarchical assembly (Fig. 18.1) overcomes the fundamental incompatibility between buckle-like and rod-like cellulose fibers, using hemicelluloses combine at the cellulose/lignin contact.

To release cellulose fibers, so-called pulping methods must be applied either mechanically or chemically. Before being split to fibrous material through abrasive refinement or grinding, mechanical pulping includes treating wood with steam. To dissolve the lignin and other compounds of the plant's material, chemical pulping is mainly dependent on chemical reactants and heat and mechanical refinement for separating the fibers. These two techniques are mostly used to manufacture fiber material industrially (Sixta 2006). The worldwide yearly pulp production in 2010 came to almost 4 to 10^8 tones (Toland et al. 2002).

18.2.1.2 Cotton Linters

Cotton (*Gossypium* spp.) is an annual shrub that grows in the subtropical and tropical regions of the world, both north and south of the equator. The seed capsules of cotton blossoms contain 30–40 oil-containing seeds. Each cottonseed can produce anywhere from 5000 to 20,000 single seed hairs, or cotton fibers (Baumwollbörse

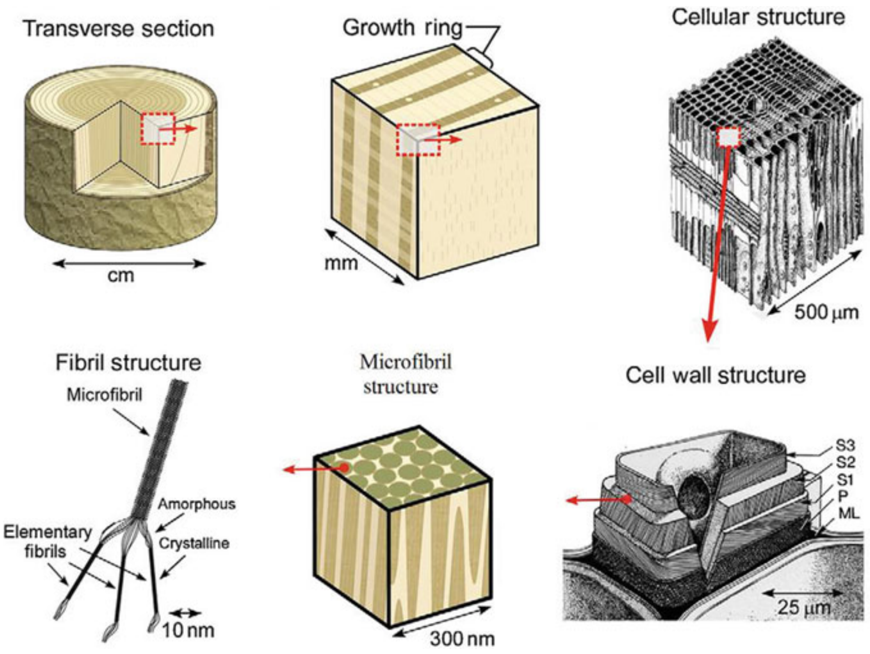

Fig. 18.1 Structure of elementary fiber and simplified cellulose organization in hierarchical order from wood to the cell wall. *Source* Nasir et al. (2017a, b)

2008) (Fig. 18.2).Lint and linter are two different types of lint. The long-fiber population is represented by lint (staple cotton). Cotton linter is the short and thick-walled fibers of the fuzz (Temming et al. 1973).

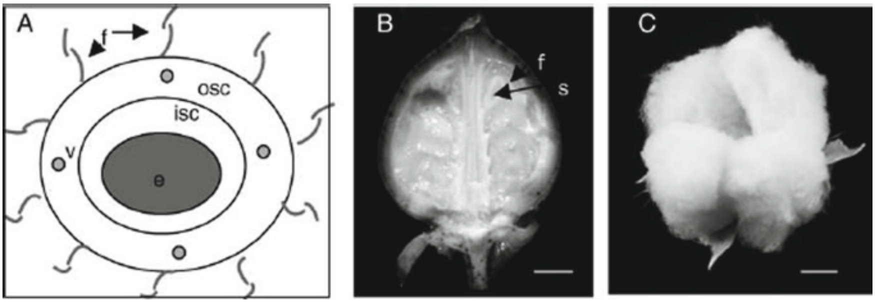

Fig. 18.2 The anatomy of the developing cotton seed and fruit. (A) A schematic representation of developing cotton seed. (B) A longitudinal section of a cotton fruit at 6 d after anthesis (DAA). Note the young fibers from the seed epidermis inside the fruit. Bar ¼ 0.5 cm. (C) A fully mature cotton fruit at about 60 DAA, showing massive amounts of long fibers. Seeds are covered by fibers. Bar ¼ 1.3 cm. Abbreviations: e, endosperm and embryo; f, fiber; isc, inner seed coat; osc, outer seed coat; s, seed; v, vascular bundle. *Source* Ruan (2005)

Cotton linter typically contains 80% cellulose on a bone-dry basis (Rafiq Chaudhry and Guitchounts 2003; Temming et al. 1973). The inherent and non-natural contaminations in cotton linter cellulose are eliminated using a bleaching process, resulting in cellulose of extremely high purity. Bleaching is a series of mechanical and chemical cleaning procedures. After the bales are opened, they are cleaned to eliminate physical contaminants such as field litter (through dry cleaning), sand, stones and seed hulls (via wet cleaning). Natural contaminants such as pectin, proteins and lipids are reduced as a result of wet cleaning. The digestion of caustic soda is the first stage of chemical purification. Fats and waxes are saponified, while breakdown products, pectin and proteins are dissolved in an alkaline media.

Purified cotton linter has a high DP (when compared to most wood pulps) and is distinguished by its high purity, high a-cellulose content and high crystallinity. They are lignin-free and have a low number of carbonyl and carboxyl groups. As a result, using cotton linter in cellulose chemistry produces excellent yields, products that are resistant to light, heat and aging (for cellulose acetates) and derivatives that create clear, transparent and colorless high viscosity solutions. In the case of fiber manufacturing, the dissolved biopolymer has good filterability and spinnability, when using the cuprammonium and viscose process (Heinze et al. 2018).

18.2.1.3 *Alternative Sources of Cellulose: Sisal and Agricultural Residues*

The driving force behind the growing interest in the extraction of cellulose from lignocellulosic by-products in agriculture is clear: the demand for wood in the construction, furniture, fiber, pulp and paper industries is growing from 1 up to 2% per year (Chandra 1998). Agricultural by-products, such as rice and wheat straw, corn cobs and bagasse, are generated in very large quantities, which represent an environmental and health problem, for example, when they are burned on the surface. Outdoor land is, on a large scale, an economic asset, if converted into material of greater economic value, for example, cellulose, animal feed and biofuels.

18.3 Structure of Cellulose

18.3.1 Cellulose Chemical Structure

Cellulose is a linear homopolymer consisting of D-glucopyranose units (also known as anhydrous glucose and glucopyranose units but often abbreviated AGU) linked by β1,4 glycosidic linkages. It mainly contains carbon (44.44%), hydrogen (6.17%) and oxygen (49.39%). The chemical formula of cellulose is $(C_6H_{10}O_5)_n$; n, called the degree of polymerization (DP), which represents the number of glucose groups, ranging from hundreds to thousands or even tens of thousands. During the twentieth century, it was shown that cellulose consists of purified repeating units of dehydrated

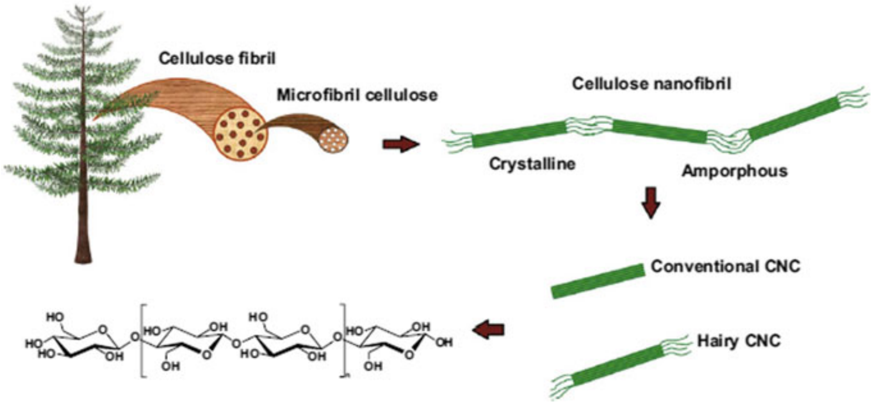

Fig. 18.3 Schematic representation of cellulose structures from resources to molecular level. *Source* Tavakolian et al. (2020)

D-glucose, as shown in Fig. 18.3 and that the repeating units of cellulose are called cellobiose (Zugenmaier 2001).

In the β-anomer, the hydroxyl group at C1 is located at the equatorial position, while in the α-anomer, the hydroxyl group is located at the axial position. The relative stability of the two anomers depends on the environment (Clayden et al. 2001).

Sodium hydroxide solution of different concentrations and temperatures can dissolve cellulose of different DP. According to the different solubility under certain conditions, cellulose can be divided into three categories: (1) α-cellulose, which is dissolved in 16.5% NaOH at 20 °C, (2) β-cellulose, which separates the acid solution and the remaining alkaline solution after neutralization Extraction and (3) gamma cellulose, which is a soluble residue in the neutralization solution. Staudinger uses the viscosity method to measure the DP of these three celluloses. The results showed that the DPs of α-cellulose, β-cellulose and γ-cellulose were greater than 200, 10–200 and less than 10, respectively. The industry usually uses α-cellulose to express the purity of cellulose. Traditionally, β-cellulose and γ-cellulose are collectively referred to as industrial hemicellulose. Whole cellulose refers to all carbohydrates in natural cellulose materials and is also the sum of cellulose and hemicellulose (Shuhui 2001).

18.3.2 Cellulose Physical Structure

The physical structure of cellulose refers to the spatial arrangement of structural units of various scales, including the chain structure and aggregation structure of polymers. Chain structure, also called primary structure, represents the geometric arrangement of atoms or groups in a molecular chain. Aggregate structure, also known as secondary structure, refers to the internal structure of the entire polymer, including crystalline structure, amorphous structure, oriented structure and liquid crystal structure. The chain structure of a polymer is the most important structural level, which reflects many properties of the polymer, such as melting point, density, solubility, viscosity and adhesion. The aggregate structure of the polymer is the main factor that determines its performance. Products are made from macromolecular compounds (Zhan 2005).

18.3.2.1 Filament Structure

Fibril is a small, stretchable unit, these units polymerize and then form the structure of some natural and synthetic fiber materials (textile fibers, wood or fibrin). Also, they form long molecular chains bundled in one direction. Due to the different sizes of fibril aggregates, current terms include elemental fibrils, micro and macro filaments (microfilament bundles). Natural cellulose has 10,000 glucose units and fibrils contain approximately 60–80 cellulose molecules. Hydrogen bonds are formed between adjacent molecules. In a certain area of space, when a certain number is reached, hydrogen bonds can be displayed in the X-ray image. This space is called the crystalline region, and the rest is called the amorphous region. Microfilaments are composed of basic fibrils and have a fixed size. The filament is composed of more than one microfilament and its size varies with the source of raw materials or processing conditions (Gao and Tang 1996).

18.3.2.2 Aggregation Structure

The aggregation state of cellulose, also known as the supramolecular structure of cellulose, mainly refers to how the cellulose molecules are arranged to form a crystalline and amorphous structure and then the basic fibril, fibril and microfiber structure. X-ray diffraction studies have shown that in the aggregates of cellulose macromolecules, the molecules are regularly arranged according to the crystal structure and have a clear X-ray pattern, which makes the cellulose density in the crystalline region high (1.588 gcm^3). The molecular chains in the amorphous region are arranged irregularly and loosely, which makes the distance between the molecules large. The density of cellulose in the amorphous region is lower at 1,500 gcm^3. However, the molecular chain is almost parallel to the main axis of cellulose. The crystallinity of cellulose, usually between 30 and 80%, refers to the percentage of all cellulose taken up in the crystallization zone (Shuhui 2001). Cellulose crystals are polymorphic. There are five crystal forms of solid cellulose and its properties can be reflected by the properties of its unit cell. Cellulose crystals can be transformed into many crystal variants under certain conditions. Type I is the crystalline form of natural cellulose. Type II, Type III, Type IV and Type X are those crystal forms of artificial cellulose under artificial processing. The generally accepted type I-unit cell structure is the monoclinic unit cell model introduced by Meyer and Misch in 1937 (Zhan 2005).

It was first discovered by Payen in 1965 and is widely used as a mechanical strength-promoting substance (Barja 2021; Zhang et al. 2020). Its derivatives are widely used in various industries. Production of cellulose biopolymers is usually limited to 6109 tons annually. Although cellulose is a crystalline structure, its crystallinity is imperfect. This means that a portion of the cellulose chain is less ordered than the ordered regions. The degree of crystallinity depends on the origin and isolation method. Hemicellulose and lignin are the main components of cellulose, which is bound together by microfibrils. These microfibrils have a width of 10 to 50 m (He et al. 2016; Meng et al. 2016).

Cellulose is an abundant organic material on earth that has renewability, biodegradability and non-toxicity. It is produced by photosynthesis. Various sources of cellulose include algae, fungi, marine animals, and bacteria. These include biomass from lignocellulosic forests. Cellulose has multiple structural levels as shown in Fig. 18.4. Its chain length is determined by the number of glucose units. Individual cellulose chains are assembled through van der Waals forces and hydrogen bonding (Köklükaya et al. 2017). These components then join into micro-fibrillated cellulose.

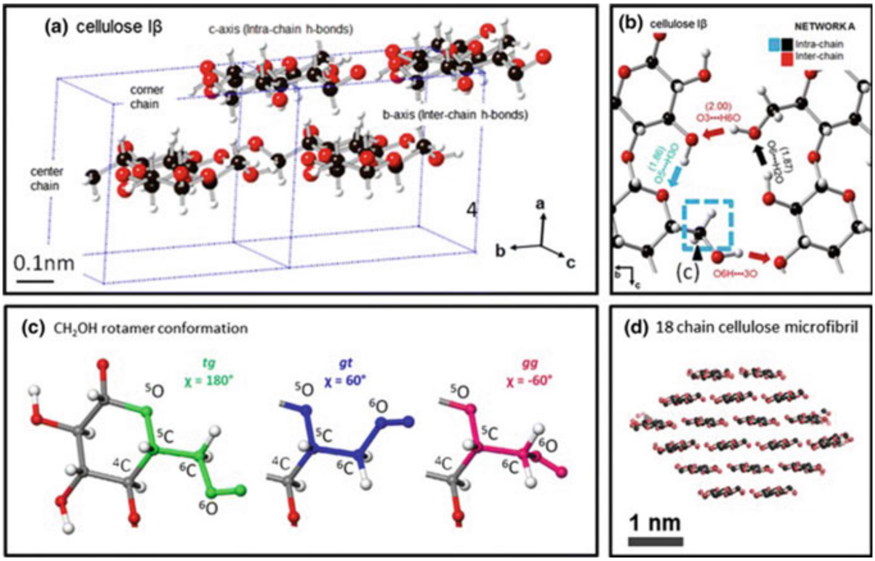

Fig. 18.4 **a** Perspective representation of two-unit cells of cellulose Iβ determined from X-ray and neutron diffraction studies. The cellulose chain axis is along the c-direction and the hydrophobic facets of the two alternate sheets are stacked along the a-direction. The C, O, and H atoms are represented as black, red and light gray, respectively, **b** Hydrogen bonding network with the perspective looking down the *bc* plane. The hydrogen-bonding network contains three hydrogen bonds 2O–H···6O (black), 3O–H···5O (blue) and 6O–H···3O (red), **c** Three possible conformations, *tg*, *gt* or *gg*, of the OH group in the exocyclic C6H$_2$OH group are shown on the right glucose unit. The *t* and *g* characters stand for *trans* and *gauche* conformations, respectively. The first italic character refers to the relative position between O5 and O6 (O5–C5–C6–O6), and the second italic character refers to the relative position between C4 and O6 (C4–C5–C6–O6). χ is the angle between C5–O5 and C6–O6 bonds and **d** Cross-section view of a recent 18-chain model for cellulose microfibrils. *Source* Makarem et al. (2019)

Different sources of cellulose, different isolation methods and different structural structures have led to different classifications of the commodity. Due to the rapid development of nanotechnology, many studies are focused on the isolation, characterization, and application of nano-cellulose. This bio-based material is mainly used as a component of food packaging. There are four polymorphs of cellulose, namely, cellulose I, II, III, and IV. The main differences between these four types of cellulose are their structure and properties.

According to the fringe-micelle theory, cellulose is a semicrystalline polymer that has several crystalline and amorphous parts. It is formed by the addition of a building block unit d-glabranose. The repeating unit in cellulose is composed of two glucose molecules known as hydro cellobioses. The disaccharide or DP of native cellulose varies depending on the source of the cellulose. Cellulose I is native to the allomorphs I and I. It is also known as regenerated cellulose. This type of crystal is stable and can be obtained by ammonia treatment (Kumari et al. 2021).

Cellulose is the most abundant raw material on earth. Its annual production is around 1010 t. This resource is mainly utilized in the paper industry. In terms of sustainable production, cellulose is the most abundant raw material on earth (Ho et al. 2020; Siqueira et al. 2019). It is estimated that the annual production of this commodity is around 1010–1011 t. However, only a small portion of this resource is used by the industrial sector.

This biomacromolecule was first extracted by Payen (1938), and its chemical structure was revealed by Herman Staudinger in 1941. These groups are involved in the production of micro-fibrils that are then formed into macro-fibrils. The properties of cellulose are related to the degree of polymerization and the length of the cellulose chain (Hoenders et al. 2018). Depending on the source and the extraction procedure, its crystallinity can vary from 40 to 70%. It is also prone to react with other groups. In contrast, crystalline ones are more resistant to various mechanical and chemical treatments. Various sources such as grass, wood, and animal feed can be used as raw materials to produce cellulose. A graphical representation of cellulose's molecular structure and properties is shown in Fig. 18.5.

18.4 Nomenclature and Types of Nanocellulose

Nanoscale technology has become one of the key factors that are driving a new industrial revolution in various fields, such as bio nano composites, sensing and biosensing. Despite its widely studied nature, nano cellulose has been regarded as a promising material in the last two decades. According to numerous widely used databases, it has various useful features such as its high surface area to volume ratio, low thermal expansion, and biocompatibility (Zhai et al. 2016).

Nanocellulose is a new family of nanomaterials that has wide applications in various materials-related domains. Its various properties can be affected positively. At an initial workshop held in Arlington, the terminology for nano cellulose was

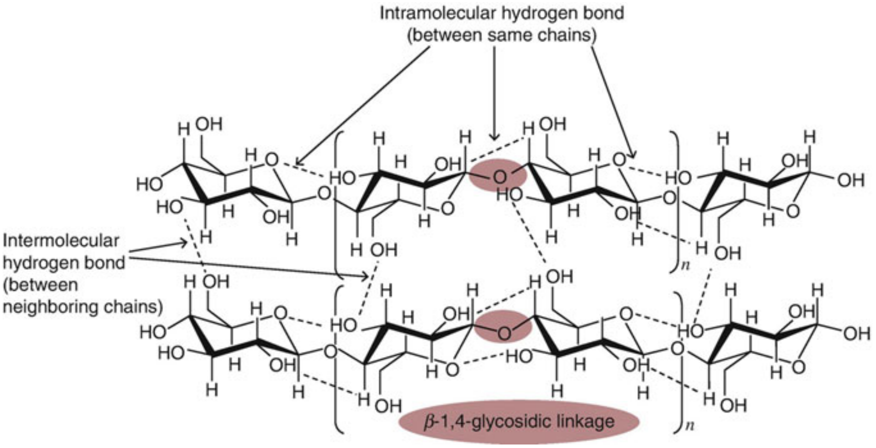

Fig. 18.5 Graphical representation of cellulose's molecular structure and properties. *Source* Ciolacu (2018)

suggested to avoid confusing the public. For instance, cellulose nanofibrils are classified as separate materials from cellulose microfibrils (Wang et al. 2017).

Although bacterial cellulose is not considered a type of nano cellulose, it is still not included in this review. Instead, it is classified into two groups viz., nanocrystalline cellulose and nano-fibrillated cellulose (Zhai et al. 2016). To promote the use of cellulose nanomaterials, the Technical Association of the Paper Industry established a division dedicated to defining the terms for these natural fibers. The organization has already established a draft version of a standard that will be used by the industry.

The first two categories are microcrystalline cellulose and bacterial cellulose. The former mainly consists of cellulose nanocrystals and cellulose nanofibril (Fedorov et al. 2019). Throughout the last two decades, various groups have been called nanocrystals, rods like cellulose crystals, nanorods, cellulose nanoballs and cellulose whiskers. These terms have also evolved to include cellulose nanocrystals and nano cellulose (Subhedar et al. 2021).

Nano fibrillates cellulose, often obtained by mechanical processing, is a network structure with flexible and long nanofibers. It exhibits low crystallinity and is usually obtained using cellulose nanocrystals (CNCs). Cellulose nanofibers (CNF) are commonly found in various sources such as animal feed, medical equipment and research fields. Although it is known to be a promising biomaterial for biomedical applications, its cost-effectiveness is still not known (Wang et al. 2021).

Due to the increasing interest in the use of nano cellulose as a material for high-performance biomaterials, the synthesis of nano cellulose has achieved remarkable growth. Due to its biodegradable properties, nano cellulose is considered a sustainable material. Its various properties vary depending on the process and production method used (Mali and Sherje 2022). Positively charged sulfate groups are attached to the surface of nano cellulose particles, which prevent the aggregation of these particles in aqueous suspensions. Cellulose nanocrystals are different from cellulose nanofibrils.

These are mainly produced by the disintegration of cellulose fibers into nano-sized particles (Nasir et al. 2017a, b).

Conventional methods to produce bacterial cellulose (BC) and electrospun carbon nanofibers (ECNF) are not very successful. This process is now being studied for industrial applications. Due to the discovery of various pre-decomposition methods that can improve the mechanical separation of CNF, it has become a more widely used material (Mali and Sherje 2022). Nanocellulose is a type of cellulose that has a diameter of fewer than 100 nm. It can be divided into two categories: (1) nanofibers and (2) cellulose nanocrystals. The former has a diameter of less than 100 nm and is mainly used as a thickening agent. Individual CNC has a diameter of 3–50 nm and a length of 30–1000 nm. Cellulose nanofibers are typically produced through various mechanical disintegration methods (Lavoine et al. 2012).

Due to the high energy consumption of the mechanical process, various chemical treatments are conducted to reduce the production cost. These include the use of enzymes and TEMPO oxidation. CNF has higher crystallinity and a higher specific surface area than plants. Its high aspect ratio and low thermal expansion coefficient are also remarkable properties (Ansar et al. 2022). CNF has properties that make it a suitable candidate for various applications, such as film, hydrogel and aerogel. Depending on the type of machine that one requires, different chemical treatments can be used to prepare the CNCs. These machines can only contain crystalline regions and have rod-like shapes (Patil et al. 2022).

Compared to CNFs, CNCs have a larger surface area. Its high mechanical strength and ability to self-assemble make it more attractive for surface modification. When concentrations of CNCs reach a critical concentration, they will self-organize into liquid crystals, which will result in a chiral nematic phase. This phase can be preserved through the evaporation of water. This unique property can also be utilized in the development of sensors and coatings. Aqueous CNCs are also known to have a unique refractive index. Surface functional groups of CNCs have been studied to improve their physical properties (Subhedar et al. 2021).

18.5 Methods of Preparation of Nanocellulose

Despite being the most widely used biomacromolecule on earth, nano cellulose has received more attention due to its numerous advantages, such as its biocompatibility, high specific surface area, high crystallinity and its non-toxic properties. Due to the wide variety of characteristics of nano cellulose, its properties can be customized for specific applications (Tong et al. 2020). These materials can be used in various applications, such as energy storage, food additives, biomimetic materials and medical equipment. They can also be utilized as food additives, stabilizers and catalysts. The preparation of nano cellulose usually consists of two main stages. The first is the pretreatment of cellulose to obtain pure cellulose, while the second one is the transformation of cellulose into nano cellulose (Yu and Yan 2017).

The first stage involves the removal of nonbiodegradable compounds such as resin, terpene, and fat from the feedstocks. Hemicellulose and lignin are also eliminated from these derivatives. The second stage is dedicated to the production of nanocrystals. The chains of disordered regions along the elementary fibrils are prone to hydrolytic action due to the reduced kinetic factors and steric hindrance. After the cellulose fibrils are cleaved, the resulting short CNCs with high crystallinity are produced (Laitinen et al. 2017).

Although acid hydrolysis is the oldest process used for preparing CNCs, it is also commonly utilized for various industrial applications. This process involves the use of sulfuric acid as well as various other chemicals. Although its surface is generally covered in sulfate esters, CNCs retain their thermal stability. They can also be well-dispersed in aqueous media (Mohammadi et al. 2017). In recent years, various industrial chemicals such as hydrogen, nitrogen, and organic acids have been used in the production of CNC machines. Although the use of hydrochloric acid for cellulose hydrolysis produces better thermal stability, it can also cause agglomeration due to the lack of repulsion force. This issue can affect the properties of the finished products (Rajala et al. 2016).

As part of their efforts to improve the characteristics of nano cellulose by producing CNCs, various preparation methods have been presented. These include the use of chemical acid hydrolysis. Various industrial procedures such as mechanical treatment, oxidation methods, enzyme hydrolysis, and ionic liquid treatments have been studied (Zhai et al. 2016). Various approaches have been proposed to produce CNCs. These include combining two or many processes, which can improve the properties of the CNC and reduce the cost. In addition, they have highlighted the increasing industrial interest in the field of cellulosic nanomaterials, especially due to the harmful effects of toxic chemicals on the environment and human health (Rohde et al. 2017).

Most of the time, they refer to the various steps involved in the isolation and derivatization of particles. However, they also address the issue of scaling up the process to bulk-scale. Despite the obstacles that remain, more efforts are being made to overcome them. For instance, some companies that produce finished products are preparing to expand their operations by equipping their CNC machines with the necessary capacity to handle large batches (Jiang and Hsieh 2017).

Despite the existence of alternative sources of cellulose for making CNCs, the utilization of these materials is still limited. As a result, the prices of these nanomaterials are expected to decrease significantly. Despite the lack of sufficient resources to produce large-scale CNCs, the use of alternative sources remains restrained. As a result, the price of these nanomaterials will likely decrease in the future (Deng et al. 2021).

18.6 Characterization and Properties of Nanocellulose

Due to their large surface area and the presence of several hydroxyl groups, nano cellulose is an excellent platform for surface modification. During the 1920–1930s, hydrophilization of cellulose was achieved through esterification reactions. Over the last decades, various strategies have been utilized to achieve hydrophobicity (Cindradewi et al. 2021). Although the hydrolyzation of nano cellulose is widely explored, studies related to its advanced functionalization are still in their early days. Various long-chain aliphatic compounds have been grafted onto CNFs and resins to improve their moisture absorption and interfacial affinity (Ning et al. 2017).

Due to the emergence of more sophisticated cellulose derivatives, their applications have started to be explored. One of these is the development of new products such as artificial skin and biosensors. The concept of ion exchangeable carboxyl groups using TEMPO mediated oxidation has been employed by several authors. This method is widely recognized as a promising platform for the development of various products such as emulsifiers, carbon dot anchoring, and fluorescent sensors (Li et al. 2020).

The preparation of total organic carbon (TOC) has allowed the possibility of creating new functional materials. In addition, the process can be modified by grafting or two-step methods (Li et al. 2020). The former involves the use of vinyl monomers to mechanically attach functional groups to nano cellulose. Physical and chemical cross-linking have been utilized for the improvement of various characteristics of nano cellulose-based materials. These include their moisture sensitivity and the development of flexible aerogels (Ning et al. 2017).

Nano crystallites of naturally generated cellulose have the same lateral width as the elementary cellulose nanofibrils. However, their length can vary from 50 to 200 nm. Heterogeneous acid hydrolysis promotes the formation of nanoparticles and higher-quality crystallinity cellulose. However, treating the cellulose with solvents or saturating it with liquid ammonia can reduce its crystallinity and size (Das et al. 2022a, b).

Currently, the structural organization of cellulose two-phase models is presented. However, studies on Para crystalline fractions revealed the presence of non-crystalline domains. Crystallites with Para crystalline fraction are integral components of the elementary fibrils. They are known to have the CI allomorph with either the triclinic P1 or the monoclinic P21-space group (Patil et al. 2022).

It was previously believed that elementary nanofibrils had a constant lateral width of 3.5 nm. However, recent studies revealed that the lateral size of these nanofibrils is different in different celluloses. The lateral size of elementary nanofibrils can vary widely depending on the type of cellulose. For example, the length of the elementary fibrils can reach several microns (Das et al. 2022a, b; Patil et al. 2022).

Various models of elementary cellulose were proposed to visualize the molecular structure of the cellulose. Although these models have historic value, they are not suitable to explain the properties of cellulose and nano cellulose. The model that follows shows that elementary nanofibrils are composed of non-paracrystalline nanodomains and nano-crystallites that are arranged along the fibril's inner portion (Ansar et al. 2022).

The main difference between elementary and non-paracrystalline cellulose is that the former has highly ordered cores and inaccessible constituents. The proposed model allows the study of various biochemical and mechanical properties of cellulose. For example, enzymes and acids can only be performed in weak non-paracrystalline domains of cellulose. Also, the para-crystallites of elementary nanofibrils can be easily decontaminated despite their inaccessible layers (Yu et al. 2020). The orientation of the para-crystallites can also affect the phase transitions and distortions of nano-crystallites. According to thermodynamics, the free energy from the surface can cause the nano-constituent to have an excess of free energy. Due to the nature of the nano-constituents, various of them tend to aggregate with the formation of larger structures (Kumari et al. 2021).

The degradification of plant materials through the use of plasticizing mediums can promote the cleaning of the surface of nanocrystallites and nanofibrils. An example of this is the formation of large crystal aggregates in cellulose after the hydrolytic reaction of dilute mineral acids with the cellulose (Lavoine et al. 2012). To prevent the aggregation of the released nano constituents, special technology methods are required. The aggregated elementary nanofibrils are then formed into microfibrils that are composed of layers of cellulose fibers. Cellulose is a bio-based material that can be used as the main source of nanomaterials. Its unique structure promotes the isolation of free nano constituents (Wang et al. 2021).

Due to the nature of cellulose's structure, various artificial forms of nano cellulose have been studied in the past. Some of these include nanocrystals, nanofibrils and amorphous nano cellulose. Currently, the research related to the artificial forms of cellulose is still in its infancy. Aside from being studied, these are also subject to various industrial processes (Barja 2021). In terms of cosmetic use, the powders and dispersions of cellulose nanofibrils can be used as part of the process of making cosmetic products. Unfortunately, the high energy consumption involved in the mechanical disintegration of cellulose fibers into nanofibers is a major obstacle to the commercialization of CNFs. Fortunately, various steps can help minimize this issue (Jorfi and Foster 2015).

Amorphous nano celluloses can be obtained by undergoing acid hydrolysis to produce spherical nanoparticles. These can be used for filling liquid systems and reinforcing polymer materials. Aside from being used as a thickening agent, nano cellulose can also be used as a bio-based material for various applications. In addition, its porous nature makes it an excellent alternative for producing various filtering and blotting materials (Zhan et al. 2019). Like other types of nano cellulose, the microfibrils are joined to the nanocrystals to form a network. Due to its high water content, this material is biocompatible as an implant for veterinary medicine and cosmetic applications. Nanocellulose-based foams have the potential to be used in various applications. Its low density and porous nature make them ideal candidates for various biomedical and environmental applications (Hoenders et al. 2018).

Cellulose nanofibrils and nanocrystals are two major types of cellulose nanomaterials. CNFs are obtained by mechanical refining of plant and wood fibers. CNCs are rod-like particles that are left after the acid hydrolysis of cellulose. Aside from being used as a drying agent, freeze-drying is also a widely used technique to produce low-density foams made from nano cellulose (Chen et al. 2015). This process involves removing the moisture from the product by using a technique known as sublimation. Various studies have been successfully carried out on the use of nano cellulose in hybrid foams and aerogels due to their multifunctional properties. These features can be utilized in the production of porous aerogels and foams (Azzam et al. 2017).

A recent study showed that hybrid foam made of montmorillonite and nanofibrils exhibited better thermal insulation and higher compressive strength when subjected to hydrogen bonding (Zhang et al. 2016). This material was designed to be used as an alternative to synthetic foams for food packaging. Clay-based materials such as kaolin can be commonly used as the building blocks of low-cost and sustainable materials. This resource can be found in various countries and is commonly used as low-cost and abundant clay.

The properties of kaolin and cellulose hybrid materials were also studied in non-foaming products such as paper and electronic components. The addition of kaolin helped improve the optical properties of paper. Due to their properties, cellulose nanomaterials have been extensively studied for their potential applications in water purification (Wang et al. 2017). A study conducted on aerogels revealed that they could achieve a maximum removal capacity of 86% when subjected to hydroxypropyl methylcellulose (Yang et al. 2020). The researchers noted that the surface-functionalization of these aerogels achieved a surface-active blue adsorption capability of up to 50%. A study showed that supercritical and freeze-drying techniques can be used to prepare crosslinked cellulose aerogels that are energy-efficient (Zhang et al. 2020).

Aside from nano cellulose, kaolin can also be used to decontaminate wastewater due to its ability to remove the color from the water. It can also be utilized as an adsorbent for various industrial applications. Although the use of nano cellulosic materials and clay in combination has been successfully used in the past, it is not yet clear if this process can be utilized in the production of bio-based foams. This development has the potential to accelerate the global efforts to find more effective and eco-friendly materials (Nehra and Chauhan 2021).

18.7 Applications of Nanocellulose

18.7.1 Nanocellulose and Conductive Polymers

These materials are mainly composed of carbon, metal and conductive polymers. In addition to carbon, nano cellulose can also be used to fabricate conductive composite that has high mechanical strength and flexibility. The surface grafting technique is widely used to introduce conductive materials to nano cellulose. The blending method is used to evenly distribute conductive materials among various components (Zhan et al. 2019). Currently, various materials such as conductive carbon nanoparticles and metallic nanoparticles are commonly blended with nano cellulose to create conductive hybrid materials. Green and biodegradable nano cellulose is a promising material for the electronics industry due to its properties, such as its ability to combine with conducting polymers (Subhedar et al. 2021).

18.7.1.1 Nanocellulose in Hybrid Conductive Composites

The poor solubility of certain conjugated polymers leads to the formation of brittle materials. This issue has presented new opportunities to develop eco-friendly materials with improved properties. In 1984, scientists from Lundm and Bjorklund reported the first known combination of cellulose and conducting polymers. This new material can be commonly used for sensing applications. In 2010, polyaniline was blended with chitin for humidity sensing applications. A novel technique was then introduced to make an aniline-on-chitosan hybrid (Oprea and Panaitescu 2020).

For the development of luminescent and conductive elements, they were grafted onto cellulose through a two-step oxidation process. The resulting product exhibited a varying fluorene/thiophene ratio. Studies on the development of robust and flexible conductive materials have led to the widespread use of nano cellulose (Wang et al. 2017). A recent study showed that a polypyrrole coating can be made on bacterial nanofibers using in-situ chemical polymerization. The resulting product exhibited a high conductivity and low tensile strength. Yao et al. (2017) revealed that poly (3,4-ethylene dioxythiophene)/PEDOT:PSS was used as a conductive layer on nanofibers.

The properties of the two combined materials were also enhanced using PPy and PEDOT. The researchers concluded that the resulting CNF films could now be made more energy-efficient. A well-defined core–shell structure was also used to create an interconnected network that resulted in higher electrical conductivity and better structural stability. The researchers discovered that the electrical and thermal properties of TONFC films were improved through a counter-ion exchange process instead of through a thermal exchange. The hybrid nanofilm made from nano cellulose and PEDOT exhibited higher electrical conductivity than its conventional counterpart. It also exhibited superior thermal stability (Yao et al. 2017).

The higher concentration of PEDOT: PSS enabled the creation of larger carrier densities. The resulting films exhibited high transparency of about 95%. The researchers attributed the film's improved mechanical and electrical properties to the TEMPO-mediated oxidation and the in situ photopolymerizations of a deep eutectic solvent (Zhai et al. 2016). Due to their high surface area and excellent electrical conductivity, new carbon materials such as carbon nanotube and graphene have been widely used for various applications. Although the concept of the simple blend of nano cellulose and carbon has been used for years, the hybrid approach is not suitable for commercial applications (Cui et al. 2017).

Zhai et al. (2016) studied the development of a cross-linking nano cellulose supercapacitor that could be used for various applications. The resulting product was highly resilient to compression. A step-by-step vacuum filtration technique was also used to prepare the sandwich-structured CNF/RGO films.

A study published in 2020 showed that conductive paper made from nanofiltration was able to achieve exceptional conductivity of 120 Sm1. The same process was also used to make the film. The results of the study were similar to those of traditional carbon materials. The researchers then introduced the use of amphiphilic CNF as a multi-functional material that can be used as an exfoliating and dispersing agent for graphene nanofilm (Oprea and Panaitescu 2020).

The hydrophilic surfaces of the CNF-bound films acted as carriers for the dispersed graphene. The cationic surfactants, which are cationic, stabilized the CNF-bound films in aqueous solutions. The reduction of the fractional free volume allowed the nanocomposites to exhibit higher electrical conductivity. The cationic surfactants also exhibited good stability while carrying out the same function (Cui et al. 2017).

A combination of triethanolamine and TOCNF was then used to make a hybrid nanofilm with enhanced mechanical properties and thermal stability. The hexagonal structure of boron nitride nanosheets has garnered the attention of industrial and academic researchers due to their exceptional thermal and electrical insulating properties (Oprea and Panaitescu 2020).

A two-dimensional structure known as MXenes has also attracted attention due to its metal-like electrical conductivity. Its unique surface design could allow the use of its hydrophilic properties without losing its electrical conductivity. A study published in 2019 revealed that a large number of CNFs can be combined with MXene to create a high-strength nanomaterial with good interfacial interaction (Köklükaya et al. 2017).

18.7.1.2 *Combination of Nanocellulose and Conductive Carbon Materials*

Hybrid nanocomposites made of various materials have been studied extensively. Many of these films were engineered with two or more constituent materials. The combination of these three components allowed the hybrid films to achieve high specific capacitance and outstanding retention. Due to their advantageous layered configuration, these films can be used for supercapacitor electrodes (Das et al. 2022a, b).

Aside from electrical conductivity and mechanical robustness, these features also include self-healing capability and flexibility. For their study, the researchers used a core–shell structure of TEMPO-oxidized cellulose nanofibers (TOCNFs) to create a self-healing hydrogel (Ansar et al. 2022). The hybrid electrodes made from nanohybrid hydrogels exhibited potential for the development of wearable electronic devices. The researchers stated that these hybrid nanocomposites exhibited good stretchability, flexibility and conductivity. They could be used as sensors for monitoring human activity. The researchers were able to achieve strong anchoring interactions between CNF and reduced graphene oxide (rGO) through a layer-by-layer process.

The results of this study indicated that these hybrid nanocomposites could be used for wearable applications.

In a study by Chen et al. (2015), the authors noted that the addition of graphene to the hybrid material improved its thermal stability and conductivity. The authors of this study stated that these hybrid nanocomposites could be used as supercapacitors for various biomedical applications (Chen et al. 2015).

The interactions among the components boosted the mechanical and electrical properties of the hybrid nanocomposite. The hydrogen bonds and chelation interactions among the various components boosted the mechanical and electrical properties of the hybrid nanocomposite. Due to the wide range of colors that can be emitted by different chemical structures in the visible region, the use of conjugated and non-conjugated polymers has been studied (Chen et al. 2021).

Due to the emergence of new technologies such as organic solar cells and bio-based sensors, the transition from traditional devices to flexible and foldable ones is expected to occur rapidly. Various new technologies such as organic field emission transistors, bio-based sensors and flexible displays are expected to be launched shortly (Li et al. 2022).

18.7.1.3 Nanocellulose-Based Luminescent Materials

An organic light-emitting film made from light-sensitive polymers has been presented as an alternative to metal particles and Carbon dots (CDs). Grigoray et al. (2017) showed that a light-controlled pulp fiber could be made to be invisible when exposed to ultraviolet light. However, Tong et al. (2020) demonstrated the possibility of creating a highly transparent film using free radical polymerization of cellulose. Their work has great potential for flexible electroluminescent devices.

An interesting aspect of the blue light emitted by the nanocomposite was that it responded differently depending on the strain. The same process was also used to produce a film with a variety of color variations. The blue light emitted by the nanocomposite was different depending on the strain (Beh et al. 2020). The same process was also used to produce a film with varying colors. In addition, CNC particles were added to the mix of vinyl alcohol and epoxy resins.

Nanocomposites made of rice flour and modified to have a light emission intensity response were developed to be sensor films that could detect protease activity in wound diagnosis. The robust nature of the nanocomposites enabled them to exhibit fluorescence under UV light. Their potential applications include wound diagnosis and biomedical technologies (Wang et al. 2020). The flexibility and transparency of bacterial nano paper made it an attractive substrate for the development of artificial tongues. A film made with CD grafted onto bacterial nano cellulose exhibited remarkable mechanical and physicochemical properties. An oxidizing liquid was also used as a solvent for the continuous film functionalization process. Hoenders et al. (2018) demonstrated that a film with tetrazole-functionalized CNF could be self-reporting through photo-induced phenomena.

Hybrid materials made from cellulose and other organic components have been successfully used in the production of transparent nanofilms. The films were prepared by immersing them in nitrate complexes containing sodium carboxylate groups. The properties of these films, such as their ability to retain moisture resistance and their thermal stability, have potential applications in various electronic devices (Zhan et al. 2019).

The film was able to detect copper ion interference over a wide range of metal ions. It was also highly selective when subjected to different copper ion concentrations. The resulting films exhibited flexible, thermal, and light-induced properties. Their development could provide new opportunities for temperature sensor devices (Curvello et al. 2019).

Fedorov et al. (2019) made organic films based on strontium fluoride and CNF for use as laser radiation visualizers. A similar process was used by Zhang et al. (2017b) to make transparent and flexible nanofilms. The film's thermal expansion coefficient was lower than that of fossil-based materials.

18.7.2 Application of Nanocellulose

Active packaging refers to systems that have active functions that are designed to protect products from degradation. Nanocellulose has intrinsic properties that make it an attractive candidate for the development of active food packaging. Its surface modification can endow it with new anti-bacterial and reinforcement agents (Nasir et al. 2017a, b).

18.7.2.1 Nanocellulose Antibacterial Food Packaging

According to the centers for disease control (CDC), approximately 48 million people get sick from food-related illnesses in the US each year. The agency noted that these illnesses cause around 128,000 hospitalizations and 3,000 deaths. The use of antimicrobial packaging can help prevent food-related illnesses (Beh et al. 2020). There are two types of packaging systems that are used for anti-bacterial purposes: leaching and non-leaching. The former refers to systems that are physically incorporated into the packaging. Bioactive agents, such as silver nanoparticles, are known to slowly release their nutrients to the environment to kill harmful bacteria. On the other hand, non-leaching antimicrobial packaging is made up of chemically grafted antimicrobial agents (Nasir et al. 2017a, b).

In terms of antimicrobial properties, the non-leaching system is known as active contact food packaging. Usually, bioactive agents are composed of cationic polymers that have high molecular weight. Although the direct introduction of bioactive agents is the most used method for making antimicrobial food packaging, this process can cause various issues. Aside from reducing the effectiveness of the system, the release of these agents can also contribute to air pollution (Mali and Sherje 2022).

The increasing concentration of biocides in food packaging can also contribute to the development of resistant bacteria. This issue could cause more environmental issues and health problems. Due to the numerous advantages of this method, the development of active contact food packaging has been focused on. In a study, the systems produced by these manufacturers exhibited antibacterial and bacteriostatic properties (Barja 2021). Non-toxic properties of CNCs have been observed in the development of active food packaging. They are usually incorporated into systems that are made up of hydrophilic components such as natural rubber and wheat gluten. Since the addition of 4 wt%, CNCs to a film has increased their tensile strength, it is widely used in the production of hydrophobic systems (Zhan et al. 2019).

18.7.2.2 Application of CNCs in Food Packaging

CNCs have been studied as the reinforcement agent for various packaging materials due to their non-toxicity and biodegradability. They are usually incorporated into the biodegradability and mechanical properties of various systems such as wheat gluten, natural rubber and polyvinyl alcohol (Tortorella et al. 2020).

18.7.3 Industrial Application of Nanocellulose

18.7.3.1 Nanocellulose in Paper Industry

It is estimated that around 100 million tons of cellulose are harvested annually for paper production. This process involves preparing the paper components, forming the paper board and finishing. It is also mandatory for papermakers to refine cellulose fibers to obtain strong paper (Laitinen et al. 2017; Zhang et al. 2017a). The results of studies by Leykin and Ioelvich showed that introducing nano cellulose particles to paper can increase its strength and mechanical properties.

18.7.3.2 Nanocellulose in Biomedical Industry

Due to its biodegradability, nano cellulose can be used in the bio-medical industry as a bio-compatible material for cosmetic and personal hygiene products. It can also be used as a carrier for the immobilization of various drugs and for treating various skin diseases. Its nano-size makes it a carrier that can penetrate the skin and treat different skin conditions (Curvello et al. 2019).

Advancements in nanotechnology have led to the development of new bio-inspired materials that can be used for biomedical applications. The coupling of various scientific fields has helped in the development of nano biomaterials (Brenes et al. 2021). In 2006, the first study showing the potential of nano cellulose as a biomaterial for the construction of tissues was published. This work sparked numerous research activities that are focused on the use of nano cellulose in various medical fields.

CNCs have shown great promise due to their various characteristics such as biodegradability, high surface-area-to-volume ratio and their ability to perform various mechanical and functional tasks. Although they are non-cytotoxic, CNCs can cause an inflammatory response and can induce oxidative stress in cells. Further research has revealed that their toxicity can vary depending on the surface chemistry and their size (Jorfi and Foster 2015; Kumari et al. 2021; Subhedar et al. 2021).

The effects of CNCs on the cell's immunogenicity can be modulated through their various physicochemical features. Further studies are needed to analyze the toxicity of these materials (Sharma and Bhardwaj 2019). An optimized drug delivery system has numerous advantages such as improved solubility, reduced drug release and enhanced therapeutic effect. Some of these features are exhibited by CNCs due to their high surface-to-volume ratio and low drug loading. Due to their hydrophobic properties, surface modifications are often carried out to improve the drug-binding performance of hydrophobic drugs. These modifications can be performed using the introduction of reactive functional groups on the CNC backbone (Siqueira et al. 2019).

For instance, Ho et al. (2020) and Wang et al. (2020) created hyperbranched polymers with modified CNCs by introducing glycidol as the initiator. The modified compounds were then converted into hydrazone groups and drug-delivery agents. The authors noted that an optimized colon-targeted drug release system could be developed by conjugating an anhydride CNC with a model drug and allowing the drug to be transported into the body (Siqueira et al. 2019).

In another study, the team led by Ndong Ntoutoume et al. (2016) discovered that introducing a model drug to the body through a magnetic colloid nanocrystal cluster (MCNC) could entrap it. The resulting drug-delivery system exhibited antiproliferative effects against colon and prostate cancer cells. CNCs are also known to deliver better drug delivery capacity than traditional hydrogels. In a study, researchers from China and Japan combined chitosan and CNCs to create a nanocomposite hydrogel that can transport theophylline (Ansar et al. 2022).

Wang et al. (2021) reported that such biocomposites exhibited excellent drug-controlled release behavior. They were able to successfully deliver dexamethasone through their constructs. The authors of the study stated that the development of a simple green manufacturing process could provide a highly advantageous carrier for drug delivery. This process is known as bio issue engineering. It is a promising approach to developing biological substitutes for various applications.

Due to their properties, CNC-based materials have been widely studied and are being investigated through the TE approach. This process involves carrying out various tests such as biodegradability, water retention and cell adhesion. Various techniques such as freeze-drying, solvent casting and 3D printing have been successfully used for the development of TE scaffolds. These components are known to be effective in various applications, such as the repair of various human organs and tissues (Mali and Sherje 2022).

CNCs are known to have wide compatibility with various biological materials such as chitosan, alginate and collagen. Numerous formulations have been developed and successfully commercialized in the past. Various studies have been presented to improve the mechanical and physical properties of scaffolds made using freeze-drying. The researchers noted that these components could improve the cell proliferation and adherence of the scaffolds. In another study, Curvello et al. (2019) study stated that they were able to create cross-linked CNC aerogels that can support bone tissue scaffolds. They noted that these components exhibited a flexible and porous structure. For instance, they could help promote bone growth after they are surgically installed.

Treating and managing skin injuries is an important part of a person's health care. Aside from preventing infection, a wound dressing is also an effective way to heal wounds and minimize the risk of further damage. A wound dressing should be made with non-toxic and allergen-free materials that can stimulate wound healing and minimize the spread of bacteria and toxins (Jorfi and Foster 2015). Due to their properties, CNCs have been extensively used in the wound healing industry. Numerous studies have been published on their potential to be functionalized and useful in the wound care industry (Barja 2021; Mali and Sherje 2022; Oprea and Panaitescu 2020).

18.8 Conclusions and Prospects

The development of bio-based and sustainable raw materials has been widely studied in response to the increasing global need for low-cost and renewable sources of raw materials. The energy consumed during the nano cellulose fabrication process may make it commercially impracticable for certain applications. However, this material can still be used for more complex and sophisticated projects. Due to its energy-efficient properties, nano cellulose has been identified as a promising material for the development of new energy-related devices. Due to its unique mechanical and flexibility properties, CNF has been regarded as the next big breakthrough in the field of advanced materials. Its continued development is being widely pursued by various companies.

Many of the CNF's features are known to provide excellent mechanical robustness and flexibility. Its ability to endure various load conditions has been demonstrated by using free-standing films made with the material. Through the CNC process, CNF can percolate with its surface chemistry. Its unique properties make it an attractive material for the development of functional chemicals and other non-toxic materials. Further studies are expected to be conducted on the integration of nano cellulose into society and commerce. Currently, it is focused on the development of luminescent films for sensing and detection applications. Also, it has been discovered that the

TEMPO-mediated Oxidation of CNF can be used for the development of light-emitting materials. Despite its numerous advantages, the process of making high-performance materials from renewable resources still requires the use of multiple components.

Due to its unique luminescence and conductivity, CNF has been widely considered a material that can be used for the production of green electronics. Due to the nature of bio-based materials, their development requires the use of various features and functionalities that are not found in nano cellulose. This, the use of a nano cellulose template can provide the necessary features and functionalities to enhance the performance of the finished product. Through its functional properties, nano cellulose can be used to develop new materials to produce flexible electronics and energy-efficient components. The present chapter aims to stimulate further research in the field of nano cellulose by reviewing the various steps involved in preparing and modifying nano cellulose. Although the literature on the subject has been extensively studied, some of the challenges that remain are related to the various stages of production and extraction.

It exhibits various useful characteristics such as high surface area, anisotropic shape, and better mechanical properties. Due to the emergence of cost-effective sources of nano cellulose, its potential is expected to grow even further. Further studies are needed to be conducted to establish the feasibility of the final products and to introduce them to the market. This can be done through the development of new methods and processes that can improve the efficiency of the production process. Despite the various challenges involved in making nano cellulose-based materials, we believe that their potential can improve the quality of life of people in the future.

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