



Review Article

Plant Molecular Defense Mechanisms Promoted by Nanoparticles against Environmental Stresses

Zainab M. Almutairi

Biology Department, College of Science and Humanities, Prince Sattam bin Abdulaziz University, P.O. Box: 83, Al-Kharj, 11940, Saudi Arabia

*For correspondence: z.almutairi@psau.edu.sa; zainab.almutairi@yahoo.com

Abstract

The alleviation of environmental stress on plants by the application of nanoparticles (NPs) has been a significant trend in the agricultural research of the last decade. Various sources of stress, such as salinity, drought, flooding, low and high temperatures, light, darkness, heavy metals, and microbial pathogens have been shown to be mitigated by NPs. Changes in gene expression associated with NP-based alleviation, however, have not been fully investigated, and the molecular defense mechanisms associated with the enhancement of plant tolerance by NPs need to be better understood. To this end, this paper reviews all available reports regarding plant transcriptomic and proteomic responses to NP-based alleviation of stress conditions. Despite their limited number, these reports discuss molecular defense mechanisms promoted by NPs under stress. Selected studies targeted salinity, drought, flooding, cold, heavy metal cadmium (Cd), fluoride and the antibiotic tetracycline (TC) as sources of abiotic stress, as well as biotic stress caused by pathogens in addition to wounding in plant tissues which might due to abiotic or biotic factors. From these studies, large number of genes appears to be responsive to NPs under stress, suggesting that unique molecular defense patterns arise from the combination of NP treatment and environmental stress. Moreover, transcriptional repression is shown to be the basic molecular mechanism of the plant response to NPs, regardless of type or size. This review provides an illustration of the molecular defense mechanisms promoted by NPs for plants suffering from abiotic or biotic stresses. © 2019 Friends Science Publishers

Keywords: Plant; Molecular mechanisms; Environmental stress; Nanoparticles

Abbreviations: ABA: abscisic acid, *ADH1*: Alcohol Dehydrogenase 1, AgNPs: silver nanoparticles, Al₂O₃NPs: aluminum oxide nanoparticles, *APR*: Adenosine-5'-Phosphosulfate Reductase, *APT*: Adenylytransferase, *APX2*: Cytosolic Ascorbate Peroxidase 2, *AREB*: Abscisic Acid Response, Element-Binding Protein, *BKRI*: Beta Ketoacyl Reductase 1, CAT: catalase, Cd: cadmium, *CRK1*: Cysteine-Rich Receptor-Like Protein Kinase 42-Like, *DDF2*: Dwarf And Delayed Flowering 2, *ECS*: γ -Glutamylcysteine Synthetase, Et: ethylene, *ERF5*: Ethylene Response Factor 5, GPX: glutathione peroxidase, GS: glutathione synthetase, GSH: glutathione, H₂O₂: hydrogen peroxide, *HMA3*: Heavy Metal ATPase 3, JA: jasmonic acid, *LCT1*: Low-Affinity Cation Transporter, *LSII*: Low Silicon Rice 1, *MAPK*: mitogen-activated protein kinase, MWCNT: multi-walled carbon nanotubes, *NCED3*: 9-Cis-Epoxycarotenoid Dioxygenase 3, NPs: nanoparticles, *NRAMP5*: Natural Resistance-Associated Macrophage Protein 5, *P5CS1*: Delta-1-Pyrroline-5-Carboxylate Synthetase 1, *PAP*: Purple-Acid Phosphatases, *PDC*: Pyruvate Decarboxylase 2, *PEPC*: Phosphoenolpyruvate Carboxylase, POD: peroxidase, *RBOH1*: Respiratory Burst Oxidase, ROS: reactive oxygen species, RT-PCR: real time polymerase chain reaction, RUBISCO: ribulose -1,5-bisphosphate carboxylase-oxygenase, SA: salicylic acid, SAR: systemic acquired response, Si: silicon, SiNPs: silicon nanoparticles, *SiR*: Sulfite Reductase, SnRKs: sucrose non-fermenting1-related protein kinase, SOD: superoxide dismutase, SOS: salt overly sensitive, *TAS14*: Abscisic Acid and Environmental Stress-Inducible Protein, TC: tetracycline, TiO₂NPs: titanium dioxide nanoparticles, *ZFHD1*: Zinc Finger Homeodomain Transcription Factor, ZnONPs: zinc oxide nanoparticles

Introduction

Environmental stress critically influences plant growth and production. As such, plants have evolved immune systems and defense responses that increase their tolerance to environmental stress. For the technological enhancement of agriculture, it is essential to study and understand plant tolerance mechanisms. A decisive step in a plant's response to environmental stress is its fast recognition of the stress in

order to defend against it rapidly and efficiently. After recognition, and depending on the nature of the stress, the plant's substantial defense mechanisms trigger complex signalling cascades of defense. Early response to stress involves the activation of specific ion channels, kinase cascades (Fraire-Velázquez *et al.*, 2011) and accumulation of reactive oxygen species (ROS) (Laloi *et al.*, 2004). Additionally, early response stimulates plant phytohormones like abscisic acid (ABA), salicylic acid

(SA), jasmonic acid (JA), and ethylene (Et) (Bari and Jones, 2009). This initial response also involves an activation of the gene networks, resulting in an effective defense by which the plant is protected from the injury caused by the stress (Rejeb *et al.*, 2014).

Reactive oxygen species are chemically reactive chemical species formed by the normal metabolism of oxygen. However, during exposure to stress, ROS levels increase, resulting in an oxidative stress in the cell (Allen, 1995). To cope with oxidative stress, living organisms have evolved antioxidant defense systems, which keep ROS levels under control within the cell (Apel and Hirt, 2004).

Plants respond to different types of abiotic and biotic stresses through multiple complex signalling pathways. The sensing of stress induces signalling cascades, which activates the expression of specific defense genes. The functions of proteins participated in those pathways are detoxification of ROS (Scandalios, 2005), induction of signalling cascades such as mitogen-activated kinase (MAPK) and salt overly sensitive (SOS) kinase (Fraire-Velázquez *et al.*, 2011), transcriptional control (Saibo *et al.*, 2008) and water and ion uptake and transport (Blumwald, 2000).

Nanotechnology holds the promise of enhancing plant production through the improving disease resistance and plant tolerance of challenging environments. The interactions between NPs and plants have been widely studied at the physiological and morphological levels, as both positive and negative effects have been reported. Over the past few years, the field of nanotoxicology has been dealing with the adverse effects of NPs and their interactions with plants. More recently, however, NPs have been applied as a means of mitigating the adverse effect of stresses. This mitigation role of NPs depends on NPs' size, shape, and dosage, as some concentrations are shown to be toxic for plants while lower concentrations have positive effects (Jha and Pudake, 2016; Siddiqi and Husen, 2017).

The protective effects against abiotic stress of NPs on plant germination, growth, reproduction, and yield have been extensively reported (Saxena *et al.*, 2016; Khan *et al.*, 2017). Controlling plant pathogens through NP application is also well known (Chowdappa and Shivakumar, 2013). However, the molecular mechanisms related to NPs' protective role are still unclear. Therefore, understanding plant molecular defense mechanisms shows the benefit of using NPs in protecting plants from stressors. Plant molecular response to NPs has been reviewed in recent reports (Aken, 2015; Jha and Pudake, 2016). Likewise, plant molecular responses to abiotic and biotic stresses have been also reviewed (Rejeb *et al.*, 2014). Little progress has been made, however, in revealing the effect of the combination of NPs and stresses on plant tolerance at the molecular level.

This paper reviews the available information about plant molecular mechanisms related to the role of NPs in alleviating the negative effects of stress, arranged according to stress type. A total of 12 studies (Table 1) have discussed

the changes in gene expression induced by NPs against environmental stress. All selected studies discuss the alleviation role of NPs against abiotic and biotic stresses. Thus, the studies that did not show any alleviation of adverse effect of stress by NP treatment have been excluded from this review. The 12 selected studies have targeted salinity, drought, flooding, cold, Cd, fluoride and TC as sources of abiotic stress, wounding and common plant pathogens as sources of biotic stress. In the various studies, the main NPs used have been aluminum oxide nanoparticles (Al_2O_3 NPs), silicon nanoparticles (SiNPs), silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnONPs), titanium dioxide nanoparticles (TiO_2 NPs), and multi-walled carbon nanotubes (MWCNT). Each of the selected reports has targeted only a single stressor by NP treatment. Three recent studies focused on salinity, while three others targeted flooding; two studies targeted cold and pathogens stresses; there is a single study for each of the drought, Cd, fluoride, TC and wounding. The study of García-Sánchez *et al.* (2015), however, targeted five sets of stresses with three types of NPs. Thus, the results of García-Sánchez *et al.* (2015) will be reviewed for each of the five stress types in turn. This review paper provides an overview of the plant molecular response to NPs under stress conditions, with an emphasis on the data reported through transcriptome and proteome analysis.

Environmental Stresses Targeted by NPs with Related Molecular Defense Mechanisms

Salinity

Salt stress is a considerable abiotic stress that limits crop production. It affects all aspects of plant development and reproduction. Many salt ions are toxic to plant cells, causing osmotic stress, nutrient deficiency, and oxidative stress on the plants (Bano and Fatima, 2009). The role of NPs in mitigating salinity has been widely reported, although the molecular responses related to this mitigation remain unclear. Three studies, however, have analyzed plant transcriptome in response to NPs under salinity stress. Almutairi (2016b) examines the expression of salt-stress genes in tomato (*Solanum lycopersicum* L.) seedlings that showed the best alleviation of salt stress effect under AgNP treatment. Of the examined salt-stress genes, four genes (ABA Response Element-Binding Protein (*AREB*), *MAPK2*, Delta-1-Pyrroline-5-Carboxylate Synthetase 1 (*P5CS1*), and Cysteine-Rich Receptor-Like Protein Kinase 42-Like (*CRK1*)) are up-regulated and three genes (ABA And Environmental Stress-Inducible Protein (*TAS14*), Dwarf And Delayed Flowering 2 (*DDF2*), and Zinc Finger Homeodomain Transcription Factor (*ZFHD1*)) are down-regulated by AgNPs under salt stress (Table 1). Likewise, Almutairi (2016a) investigates the change in the expression patterns of the salt-stress genes associated with mitigation of salinity damage in tomato plants exposed to SiNPs. Under

Table 1: Plant transcriptomic and proteomic response to NPs under biotic and abiotic stresses. Plant genes regulated by a combination of NPs and stressors as reported in the available studies. The column Exp. indicates for expression patterns where up- and down-regulation are represented as + and -, respectively. Differentially expressed genes represented as +/-

NPs	Plant	Stress	Major regulated genes	Exp.	References
TiO ₂ NPs, AgNPs, MWCNTs	<i>A. thaliana</i>	<i>Alternaria brassicicola</i> ; <i>P. syringae</i> pv; salinity; drought; wounding	Genes responsive for salt, <i>Alternaria brassicicola</i> , <i>P. syringae</i> pv., root hair and SAR. Phosphate starvation-responsive genes. Drought-responsive genes.	-	García-Sánchez <i>et al.</i> (2015)
SiNPs	Tomato	Salinity	<i>AREB</i> ; <i>TAS14</i> ; <i>NCED3</i> ; <i>CRK1</i> .	+	Almutairi (2016a)
AgNPs	Tomato	Salinity	<i>RBOH1</i> ; <i>APX2</i> ; <i>MAPK2</i> ; <i>ERF5</i> ; <i>MAPK3</i> ; <i>DDF2</i> .	-	
			<i>AREB</i> ; <i>MAPK2</i> ; <i>P5CS1</i> ; <i>CRK1</i> .	+	Almutairi (2016b)
			<i>TAS14</i> ; <i>DDF2</i> ; <i>ZFHD1</i> .	-	
Al ₂ O ₃ NPs	Soybean	Flooding	Proteins related to protein synthesis/degradation; glycolysis; lipid metabolism.	+/-	Mustafa <i>et al.</i> (2015a)
			<i>NmrA-Like</i> , <i>PABP2</i> .	+	
			<i>FQR1</i> .	-	
AgNPs	Soybean	Flooding	Root proteins for stress signalling and cell metabolism.	+/-	Mustafa <i>et al.</i> (2015b)
			<i>Glyoxalase II 3</i> and fermentation related proteins; <i>ADH1</i> ; <i>PDC</i> .	-	
AgNPs	Soybean	Flooding	Proteins related to protein metabolism; cell division/organization; amino acid metabolism, protein synthesis; ribosome; <i>BKRI</i> .	+	Mustafa <i>et al.</i> (2016)
TiO ₂ NPs	Chickpea	Cold stress	<i>LRubisco</i> ; <i>SRubisco</i> ; <i>Chlorophyll a/b-Binding Protein</i> ; <i>PEPC</i> .	+	Hasanpour <i>et al.</i> (2015)
TiO ₂ NPs	Chickpea	Cold stress	Genes related to cellular defense, cell signaling, transcriptional regulation and chromatin modification.	+/-	Amini <i>et al.</i> (2017)
SiNPs	Rice	Cd	<i>LCT1</i> ; <i>NRAMP5</i> .	-	Cui <i>et al.</i> (2017)
			<i>HMA3</i> ; <i>LSII</i> .	+	
AgNPs	<i>Cajanus cajan</i>	Fluoride	NADPH oxidase; <i>P5CS1</i> .	-	Yadu <i>et al.</i> (2018)
TiO ₂ NPs	<i>A. thaliana</i>	TC	<i>APT</i> ; <i>APR</i> ; <i>SiR</i> ; <i>ECS</i> ; <i>GS</i> .	+	Liu <i>et al.</i> (2017)
AgNPs	<i>A. thaliana</i>	<i>Alternaria brassicicola</i>	Proteins involved in bioenergy, metabolism; cell signalling; storage; biogenesis; miscellaneous functions.	+/-	Kumari <i>et al.</i> (2016)

salt stress, four salt-stress genes (*AREB*, *TAS14*, 9-Cis-Epoxyxycarotenoid Dioxygenase 3 (*NCED3*), and *CRK1*) are activated by SiNPs, while six genes (Respiratory Burst Oxidase (*RBOH1*), Cytosolic Ascorbate Peroxidase 2 (*APX2*), *MAPK2*, *MAPK3*, Et Response Factor 5 (*ERF5*), and *DDF2*) are repressed (Table 1). These genes, which are activated or repressed by AgNPs and SiNPs, are involved in regulating ABA pathways. The plant hormone, ABA, is known to regulate the water balance of the cell by regulating some salt-stress genes involved in stomatal function (Zhu, 2002).

The ABA signalling pathway relies basically on the activation of sucrose non-fermenting I-related protein kinase (SnRKs) to mediate gene regulation, stomatal closure, and plant growth arrangement. The plant response to ABA via SnRK2s pathways regulated by direct phosphorylation of many downstream proteins, such as RBOH and AREB. The transcription factor AREB is required for the expression of various genes in ABA pathway. However, the contribution of SnRKs to ABA-related responses is not clear (Kulik *et al.*, 2011). Generally, ABA regulates osmotic stress-responsive gene expression mainly through AREB1 transcription factors. This function for AREB is also shown during seed germination and plant early growth (Guiltinan *et al.*, 1990). Other salt stress genes, such as *NCED3* and *TAS14*, are known to be essential in ABA signalling pathways. The *NCED3* gene synthesizes ABA upon osmotic stress (Thompson *et al.*, 2000) and *TAS14* is found to increase ABA after plants perceive drought stress (Muñoz-Mayor *et al.*, 2012). Moreover, several pathways independently respond to environmental stresses through

both an ABA-dependent and an ABA-independent manner (Bartels and Sunkar, 2005). The transcription factor *ZFHD1* gene regulates defense against stress through ABA-independent pathway (Tran *et al.*, 2007). This gene is found to be down-regulated by AgNPs in Almutairi (2016b). On the other hand, *AREB* and *NCED3* are involved in the ABA-dependent pathway. The up-regulation of *AREB* and *NCED3* and down-regulation of *ZFHD1* by NPs that observed by Almutairi (2016a, b) suggests that plants tolerate stress via the ABA-dependent pathway in response to NPs.

Plant NADPH oxidases, RBOHs, are the most studied enzymatic sources of ROS. These RBOH enzymes are the core of the ROS signalling network of cells. Under stress, RBOHs integrate various signalling factors in plants (Suzuki *et al.*, 2012; Kadota *et al.*, 2014). The repression of the *RBOH1* gene by NPs, observed in Almutairi (2016a, b) studies, might be because SnRKs regulates ABA signalling via phosphorylation of AREB protein, not RBOH1. It is noteworthy, too, that the *APX2* gene that is down-regulated by SiNPs in Almutairi (2016a) is responsive to ABA and chloroplast-sourced ROS under high light and drought stress (Rossel *et al.*, 2006). Although ABA is necessary for the induction of *APX2*, it is not sufficient. The ABA signalling network that regulates *APX2* expression is accelerated by photosynthetic electron transport and H₂O₂ (Chang *et al.*, 2004). Furthermore, of those genes found by Almutairi (2016a, b) to be up-regulated by NPs, *CRK1* is involved in direct ROS sensing due to the redox regulation possibilities in its extracellular protein domain (Idänheimo *et al.*, 2014).

Interestingly, AREB1 upregulates *ERF5* and *P5CS1* in

order to tolerate stresses (Liao *et al.*, 2008). The P5CS1 is a central enzyme in the proline biosynthesis that accumulates in plants under stress conditions (Hong *et al.*, 2000). Besides its role in osmolyte adjustment, proline also acts as a metal chelator, an antioxidant, and a signalling molecule during stress (Ashraf and Foolad, 2007). The activation of genes involved in ABA signalling and ROS sensing and reduction, such as *CRK1* and *P5CS1* by NPs in Almutairi (2016a, b), reflects the NPs' role in alleviating oxidative stress through the ABA signalling pathway. These results confirm that regulating ABA signalling is one of the possible mechanisms that is triggered by NPs (Hao *et al.*, 2016; Vankova *et al.*, 2017).

Signalling pathways in response to stress include various hormones, SOS, MAPK cascade, and ROS. The SOS pathway is one of the possible signalling pathways that involves in ion homeostasis and salt tolerance (Miller *et al.*, 2008). The MAPK cascade is a signal transduction module that transfers the information from sensors to responses. In plants, MAPK is involved in the regulation of the antioxidant defense system under stress conditions. In Almutairi (2016a, b), the *MAPK2* and *MAPK3* genes are repressed by SiNPs, although *MAPK2* is activated by AgNPs under the same salt stress conditions. The repression of *MAPK2* gene by SiNPs confirms the previous suggestion that salt stress defense is activated via the phosphorylation of AREB by SnRKs protein in the ABA-dependent pathway (Kulik *et al.*, 2011).

The salt stress genes, *ERF5* and *DDF2* are shown to be repressed by NPs in Almutairi (2016a, b) studies. These genes are known to be involved in plant defense response through hormonal pathways different from ABA. The transcription factor *ERF5* is known to regulate the expression of stress defense genes. It mediates the tolerance to biotrophic and hemibiotrophic pathogens through SA signalling, while also mediating tolerance against necrotrophic pathogens through JA/Et signalling (Glazebrook, 2005). Likewise, expression of *APX2* in local and systemic tissue might be regulated by the coordination between Et and SA signalling during systemic acquired acclimation (Gordon *et al.*, 2012). Moreover, *DDF2* makes the plants more tolerant to high salinity levels by reducing gibberellic acid biosynthesis, the plant growth hormone (Magome *et al.*, 2008).

Another study by García-Sánchez *et al.* (2015) evaluates changes in *Arabidopsis thaliana* transcriptome after treatment with TiO₂NPs, AgNPs, and MWCNT, under five stress inducers using microarray and RT-PCR techniques. Saline, drought, and wounding were used as abiotic stresses, and two types of pathogens were used as biotic stress sources. A total of 351 genes that has regulated by all NPs, regardless of particle size or type, is identified. Out of these 351 NPs-responsive genes, 34 genes are responsive to salinity and all are repressed by NPs. The three types of NPs demonstrate the same expression patterns and less effect on gene expression in comparison with the

five stress inducers. According to transcriptome analysis, salt stress has lesser effects than biotic stress, although still greater than that of NP treatment. The most regulated genes are phosphate starvation-responsive genes, of which 19 genes are strongly repressed in all treatments of NPs. Purple-Acid Phosphatases (*PAP14*, *PAP17*, and *PAP24*) are among the phosphate starvation genes that are repressed by NPs. *Purple-Acid Phosphatases* are known to stimulate phosphatase activity and metal ion binding in plants, in addition to its role during oxidative stress induced by H₂O₂. These genes are responsive to phosphate starvation and are also induced in response to ABA and salt stress (Pozo *et al.*, 1999). Phosphate starvation is a common protective mechanism to different types of stresses that allows plants to cope with the nutrient deficiency (Lu *et al.*, 2014). Furthermore, 18 of the 19 genes involved in root hair development are also repressed in all NP treatment. Phosphate starvation genes and other stress signals genes are involved in development of root hairs which necessary to increase nutrient and water absorption (Lu *et al.*, 2014). During stress, roots structure and function altered to protect plants from severe damage. These alterations include changes of metabolism and membrane properties, strengthening of cell wall, and shortening of root (Gowda *et al.*, 2011). These modifications are controlled by single or integrated abiotic stress-responsive pathways (Petricka *et al.*, 2012). The repression of root hair-specific genes by NPs points to an important role of NPs on the root hairs proliferation.

Drought

Drought is a serious environmental stress that influences the cellular and physiological activities of plants and negatively affects plant development. Plants avoid drought by closing the stomatal pores that control water loss from the leaves, and by maintaining root water-uptake (Hirt and Shinozaki, 2004). The only available study that discusses the molecular mechanisms associated with NPs' mitigation of drought stress is García-Sánchez *et al.* (2015), which treated *A. thaliana* plants with TiO₂NPs, AgNPs, and MWCNT under five stress inducers, including drought. As measured by transcriptome analysis, drought stress has lesser effects than biotic stress, although still greater than that of NP treatment. Out of 351 NPs-responsive genes, 16 genes are responsive to drought. The expression of drought-responsive genes is increased in response to all tested NPs, despite the fact that biotic and salt-responsive genes are repressed in the same experiment. This activation pattern of drought-responsive genes suggests that plant regulate gene expression through an alternative mechanism, different from transcriptional repression, such as microRNAs (Burklew *et al.*, 2012; Frazier *et al.*, 2014). MicroRNAs are an important gene silencing mechanism that regulates plants' responses under stress conditions (Sunkar, 2010).

Flooding

Flooding constitutes a major abiotic stress inducer that has a critical effect on plant growth and development. Submergence of soil replaces the air in soil pores, creating a lack of O₂ around plant roots. This situation influences all physiological processes in plants, causing them severe damage (Kozłowski, 1997). Three studies analyzed transcriptome and proteome of the soybean (*Glycine max* L.) plant after exposure to NPs under flooding stress, all demonstrating that AgNPs and Al₂O₃NPs can lessen the adverse effects of flooding stress. Mustafa *et al.* (2015a) exposed soybeans to Al₂O₃NPs, ZnONPs, and AgNPs. They have found the best alleviation of flooding effects is with Al₂O₃NPs (Table 1). Proteome analysis has revealed that 172 common proteins significantly have changed in expression among non-treated plants, plants under flooding, and plants exposed to Al₂O₃NPs under flooding. Most of these proteins are related to energy metabolism, which decreases under Al₂O₃NPs as compared to flooding stress. Genes that induced in response to Al₂O₃NPs are related to glycolysis, lipid metabolism, and protein synthesis and post-translational modification. With a lack of O₂, glycolysis and anaerobic fermentation can overrun the aerobic energy metabolism (Sousa and Sodek, 2002). Interestingly, the most interactive protein in soybean root under flooding stress with Al₂O₃NP treatment is Poly(A)-Binding Protein 2 (PABP2). This protein binds the poly(A) tail of an mRNA playing an important role in promoting translation initiation (Mustafa *et al.*, 2015a). The gene, *PABP2* is a member of stress granules, which are cytoplasmic particles involved in protecting RNAs from adverse conditions within the cell during stress (Nover *et al.*, 1989; Kühn and Wahle, 2004). Transcriptional analysis for Al₂O₃NPs-responsive genes revealed that the negative transcriptional regulator *NmrA-Like* is strongly activated by Al₂O₃NPs under flooding. By contrast, the Flavodoxin-Like Quinone Reductase (*FQR1*) gene is down-regulated by Al₂O₃NPs under flooding (Mustafa *et al.*, 2015a). *NmrA-Like* is involved in signal transduction pathways (Stammers *et al.*, 2001). FQRs are detoxifying enzymes function under oxidative stress (Joseph and Jaiswal, 1994). Both *NmrA-Like* and *FQR1* are members of the *NmrA-like* family, which has strong antioxidant activity and ROS scavenging potential (Kim *et al.*, 2010). These expression patterns indicate that AgNPs combat cell death under flooding stress by improving protein synthesis and detoxifying danger molecules.

Likewise, Mustafa *et al.* (2015b) analyze transcriptome and proteome of soybean exposed to AgNPs under flooding stress (Table 1). The 107 differentially expressed root proteins are mostly related to stress signalling and cell metabolism. The expression of *Glyoxalase II 3* and fermentation-related proteins decrease in response to AgNPs by comparison with plants under flooding stress. According to transcriptome analysis, AgNPs represses the Alcohol Dehydrogenase 1 (*ADH*) and

Pyruvate Decarboxylase 2 (*PDC*) genes as compared to flooded plants. The first step of the ethanolic fermentation pathway is pyruvate catalyses by *PDC* enzyme, producing CO₂ and acetaldehyde. Then, acetaldehyde is reduced to ethanol by the *ADH* enzyme. Anaerobic respiration via ethanolic fermentation is an important mechanism for plants to cope with the lack of O₂. Under flooding stress, switching from aerobic respiration to anaerobic fermentation appears to be a common mechanism that enables plants to survive O₂ deprivation (Drew, 1997). The *PDC* gene is expressed in *A. thaliana* only during O₂ limitation, but not other environmental stresses, suggesting that the ethanolic fermentation pathway is exclusively for abiotic stresses that involve an O₂ deprivation stress (Kürsteiner *et al.*, 2003). Glyoxalase is the major pathway for detoxification of methylglyoxal into D-lactate involving reduced glutathione (GSH). The repression of *Glyoxalase II 3* after the exposure of flooded plants to AgNPs reflects the alleviation role of AgNPs by decreasing cytotoxic products to the normal levels (Hasanuzzaman *et al.*, 2017).

Another study by Mustafa *et al.* (2016) analyze the proteome of soybean in response to different sizes of AgNPs under flooding stress. Morphological data have revealed that the mitigating effect of AgNPs only occurred with size 15 nm AgNPs. The proteins related to protein metabolism, cell division/organization, and amino acid metabolism have differentially changed under varying sizes of AgNPs. Exposure to AgNPs of 15 nm repress number of protein synthesis-related proteins, while other amino acid synthesis-related proteins and ribosomal proteins are increased compared with flooded plants (Table 1). The most interactive protein under AgNPs of 15 nm is Beta Ketoacyl Reductase 1 (*BKRI*) which is up-regulated, although its enzyme activity decreases. This expression pattern of *BKRI* is shown during the flooding stress period. However, during the post-flooding recovery period, the expression of this enzyme decreases whereas its enzyme activity gradually increases (Khan *et al.*, 2015b). The main function of the *BKRI* enzyme is the biosynthesis of fatty acid and wax. Wax is the basic structure of a plant's cuticular which provides a physical barrier against several stresses (Beaudoin *et al.*, 2009). From Mustafa *et al.* (2016) results, the protective role of AgNPs under flooding appears to involve the regulation of amino acid synthesis and wax formation. Taken together, all the three reports (Mustafa *et al.*, 2015a, b; 2016) suggest that the NPs' mitigation of flooding stress appears to involve limiting cell death in roots and reducing O₂ deprivation stress via shifting from aerobic to anaerobic energy metabolism, as well as the regulation of protein synthesis and the detoxification of toxic products.

Cold Stress

Cold stress is a serious abiotic stress that causes difficulties in plant growth and production. It results in changes to the lipid component of cell membranes. This change causes the

disturbance of membrane functions, such as selective permeability and transport processes, and thereby influences essential cellular processes, such as photosynthesis. Cold stress causes inhibition in chlorophyll levels, CO₂ assimilation, transpiration rate, and degradation of ribulose - 1,5-bisphosphate carboxylase-oxygenase (RUBISCO), the enzyme responsible for CO₂ fixation during photosynthesis (Liu *et al.*, 2012). It exists as a holoenzyme composed of eight large subunits and eight small subunits. Light-induced oxidative stress known to cause damage to RUBISCO enzymes by arresting of the large subunit and subsequent degradation of the small subunit (Spreitzer, 1993).

The change in gene expression associated with mitigating effects of NPs on photosynthesis under cold has been reported in two studies conducted by Hasanpour *et al.* (2015) and Amini *et al.* (2017) (Table 1). Both studies focus on TiO₂NPs role in alleviating cold stress in two chickpea (*Cicer arietinum* L.) cultivars; cold-resistant and cold-sensitive. Hasanpour *et al.* (2015) found that TiO₂NPs caused a considerable decrease in H₂O₂ in resistant plants as compared to sensitive ones. This decrease is associated with a high capacity for photosynthesis, especially in resistant plants. Furthermore, treatment with TiO₂NPs decreases H₂O₂ content and enhances photosynthetic activity. This enhancement is shown by the abundant expression of the RUBISCO large and small subunit genes, *LRubisco* and *SRubisco*, in addition to Chlorophyll *a/b*-Binding Protein genes. TiO₂NPs significantly increases the activity of RUBISCO compared to control plants, although its activity decreases significantly under cold comparison with optimum temperature. Moreover, the higher expression of the Phosphoenolpyruvate Carboxylase (*PEPC*) gene in resistant plants compared to sensitive ones as well as in plants exposed to TiO₂NPs compared to control plants suggests a possible role of TiO₂NPs in altering energy metabolism through different mechanisms like malate (Hatch, 1987). Malate dismutation pathway is a common defense under extended lack of O₂. In the anaerobic energy metabolism, the PEPC enzyme converts phosphoenolpyruvate, generated by glycolysis, to oxaloacetate, which is then reduced to malate. This malate is imported into the mitochondrion, where it is degraded via malate dismutation (Edwards *et al.*, 2013). The up-regulation of the *PEPC* gene demonstrates the alteration from aerobic to anaerobic energy metabolism under cold stress. This alteration is common under flooding stress, as discussed above. These expression patterns indicate the protective role of TiO₂NPs in terms of photosynthesis and energy metabolism during cold stress.

The second study by Amini *et al.* (2017) investigate the alleviating role of TiO₂NPs on cold stress in two chickpea cultivars; cold-resistant and cold-sensitive, by using cDNA-amplified fragment length polymorphism technique. Out of 4200 obtained fragments, 2.62% were differentially expressed. From these fragments, 10 fragments were sequenced and found to be related to

different genes involved in cellular defense, cell signaling, transcriptional regulation and chromatin modification. Quantitative reverse-transcriptase PCR showed that six fragments of were up-regulated by cold stress, particularly on the first day of cold stress suggesting potential role of these fragments in cold defense. This expression pattern was very important to trigger cold tolerance through decreasing electrolyte leakage index content in resistant plants compared to sensitive ones. The similar effect was shown for plants that exposed to TiO₂NPs compared to non-treated plants. Amplified fragment length polymorphism from cDNA library appear to be a beneficial technique can enrich transcriptome analysis.

Cadmium

Soils in some parts of the world are contaminated by heavy metals such as Cd, copper, zinc, nickel, cobalt, and chromium. These metals constitute a serious threat in plant environments (Bell *et al.*, 2001). Heavy metals, as an abiotic stress source, cause serious damage to plants by inhibiting important physiological process (Capuana, 2011). Cadmium in a plant's environment results in oxidative damage (Romero-Puertas *et al.*, 2002), alteration of membrane function (Ouariti *et al.*, 1997), and inhibition in photosynthesis, water and nutrient uptake (Somasekaraiah *et al.*, 1992).

Just one available study discusses the molecular mechanisms associated with NPs-related mitigation of Cd toxicity in plants. Cui *et al.* (2017) demonstrate that the size of the SiNPs directly influences the Cd adsorption, the Cd/Si uptake, and the Cd toxicity in rice (*Oryza sativa* L.). They have found that the expression of the Cd uptake gene, Low-Affinity Cation Transporter (*LCT1*) and the Cd transport gene, Natural Resistance-Associated Macrophage Protein 5 (*NRAMP5*) is repressed by SiNPs. The *LCT1* and *NRAMP5* genes are known to be responsible for regulating uptake and distribution of Cd in rice (Ma *et al.*, 2015). By contrast, the expression of Cd transport into the vacuoles gene, Heavy Metal ATPase 3 (*HMA3*) and the Si uptake gene, Low Silicon Rice 1 (*LSI1*) is up-regulated (Table 1). The *HMA3* gene reduces the accumulation of heavy metals in plant roots. This reduction modulates the signalling of phytohormones involved in plant defense, such as ABA, JA, and SA (Kim *et al.*, 2014). The results of Cui *et al.* (2017) indicate that the exposure to SiNPs increases Si-uptake capacity and inhibits Cd-uptake capacity, which together results in the mitigation of Cd-toxicity. SiNPs are found to induce mitigation of Cd-stress in a size-dependent manner. Compared with the larger SiNPs size, the smaller SiNPs better repress Cd-transporting capacities and more significantly improve the Cd-tolerance. This result confirms that the Si-uptake genes can be motivated by SiNPs, and consequently induce Cd-tolerance (Verbruggen *et al.*, 2009).

Fluoride

The fluoride ion in a plant environment constitutes a source of abiotic stress that known to cause oxidative stress in plants (Thomas and Alther, 1966). Prolonged exposure and high dosage can cause severe injury to different biological aspects of plant life cycle, including germination, growth, photosynthesis and production (Thomas and Alther, 1966; Yadu *et al.*, 2016). Only one recent study has investigated the role of NPs in mitigating the adverse effect of fluoride on plants. Yadu *et al.* (2018) investigate the role of AgNPs in the mitigating of fluoride stress in pigeon pea (*Cajanus cajan* L.). Exposure of fluoride-stressed plants to AgNPs showed an enhancement in growth parameters such as germination percentage and radical length. The oxidative stress also is alleviated by exposure to AgNPs as shown from decreasing ROS, malondialdehyde and oxidized GSH. This alleviation is associated with an increase in the expression of NADPH oxidase gene and an increase of *P5CS1* gene expression. The NADPH oxidase enzymes are known to be important sources of ROS (Suzuki *et al.*, 2012; Kadota *et al.*, 2014) while *P5CS1* is a central antioxidant enzyme and metal chelator in plants (Hong *et al.*, 2000; Ashraf and Foolad, 2007). A similar expression patterns for *P5CS1* and *RBOH1* genes were shown in response to AgNPs and SiNPs under salt stress which *RBOH1* gene is one of the NADPH oxidase enzymes (Almutairi, 2016a, b).

Tetracyclines

Tetracyclines are widely-used antibiotics because of their antibacterial activities. However, these antibiotics contaminate soil and water, then accumulate in plants where they negatively affect photosynthesis, cell growth, and oxidative balance (Xie *et al.*, 2010). The interactions of NPs and cocontaminants, such as antibiotics, are not widely understood. Of the studies reviewed here, only Liu *et al.* (2017) study analyses, plant transcriptome in order to explore plant molecular defense against antibiotic stress. The study investigates the biochemical and molecular response of TiO₂NP treatment on *A. thaliana* on alleviation of TC-adverse effects (Table 1). It demonstrates that TiO₂NPs alleviates TC-toxicity by enhancing growth and decreasing oxidative stress as shown from reducing the activity of antioxidant enzymes; SOD, CAT, APX, and POD, relative to the TC-alone treatment. The expressions of Adenylyltransferase (*APT*), Adenosine-5'-Phosphosulfate Reductase (*APR*), and Sulfite Reductase (*SiR*) in the roots across all three treatments (TC alone, TiO₂NPs alone, and TC with TiO₂NPs) are strongly higher relative to the control. These three genes; *APT*, *APR* and *SiR*, are key enzymes in the sulfur assimilation pathway. The genes involved in the GSH biosynthesis pathway (γ -Glutamylcysteine Synthetase (*ECS*) and Glutathione Synthetase (*GS*)) were similarly induced in plants that exposed to TiO₂NPs or TC alone.

Sulfur assimilation in plants plays a key role in the sulfur cycle in nature. The major form of sulfur in nature is inorganic sulfate. Sulfate is reduced in the pathway of sulfate assimilation (Davidian and Kopriva, 2010). Sulfate is transported to chloroplasts (Cao *et al.*, 2013), where it is adenylated by ATP, resulting in adenosine-5'-phosphosulfate, which is reduced by the formation of toxic sulfite by APR employing GSH. The following assimilation of sulfite is catalyzed by the SiR enzyme (Nakayama *et al.*, 2000). Chelation by GSH is well known mechanisms against cadmium toxicity in plant. These peptides may bind a variety of metals in the cytosol and actively transported into the vacuole (Cobbett, 2000). GSH is catalyzed by ECS and GS, the enzymes that are known to be involved in plant tolerance to metal contamination (Mendoza-Cózatl *et al.*, 2005). Liu *et al.* (2017) results, therefore, provide an insight into the combination of NPs and antibiotics, confirming the suggestion that plants tolerate heavy metals and antibiotics via the same mechanisms (Li *et al.*, 2005).

Pathogens

Plants rely on complicated physiological processes to combat pathogen attacks. NPs represent a promising technology for their antimicrobial activity, which is shown to be highly effective against a wide range of plant pathogens. Besides García-Sánchez *et al.* (2015), only Kumari *et al.* (2016) discuss the role of NPs in eliciting plant immunity and altering plant proteome to combat pathogens (Table 1). Kumari *et al.* (2016) examine the effectiveness of AgNPs in combating black spot disease caused by *Alternaria brassicicola* in the model plant, *A. thaliana*. They demonstrate that AgNPs highly reduce disease severity and enhance plant immunity, as evidenced by significant reductions in ROS, lipid peroxidation, proline content, and the consequent decrease in stress enzymes. One hundred and seventeen differential proteins are identified with significant scores, 44% of which are involved in bioenergy and metabolism, 20% in plant defense-related proteins, while 14%, 10%, and 12% are involved in cell signalling, storage and biogenesis, and miscellaneous functions respectively. The expression levels of genes involved in protein biogenesis are enhanced as an early response of plant defense. Thus, abundant antimicrobial metabolites are produced so that the production of ROS is minimized. Consequently, the proteins involved in the late defense are down-regulated. Kumari *et al.* (2016) results suggest that the alleviation role of NPs involves decreasing oxidative stress and regulating bioenergy metabolisms.

García-Sánchez *et al.* (2015) also have found that genes involved in early plant defense signalling are up-regulated in *A. thaliana* in response to three different types of NPs, in comparison with five biotic and abiotic stress sources. Necrotizing fungus (*Alternaria brassicicola*) and bacterium (*Pseudomonas syringae* pv.) used as biotic stress sources. Out of 351 NPs-responsive genes, 141 genes are

responsive to the fungus and 114 genes are responsive to the bacterium with wide overlap between the responsive subsets. Most pathogens-responsive genes are involved in the early defense signalling which arouses systemic acquired response (SAR) to pathogen infection. All pathogen-responsive genes, including those involving in SAR via SA signalling, are significantly repressed by exposure to all three types of NPs. The phytohormone, SA, regulates plant defense to pathogens primarily through the establishment of local and systemic resistance (Loake and Grant, 2007). The exogenous exposure of SA before NP treatment in García-Sánchez *et al.* (2015) experiment prevented the inhibition in root hair formation and the colonization of distal leaves by bacteria. These effects of SA pretreatment are shown as down-regulation of root-hair-specific genes, and genes involved in SAR signalling. The plant hormone, SA plays essential role in plant development and disease tolerance. Exogenous SA is shown to enhance tolerance to biotic stress and many types of abiotic stresses (Malamy and Klessig, 1992; Khan *et al.*, 2015a).

Unlike the root hair genes, phosphate-starvation genes are up-regulated, rather than repressed under biotic stress. Many phosphate starvation-responsive genes encode cell-wall modification enzymes that shape the trichoblast, a hair-forming cell on the epidermis of a plant root (Bruxel *et al.*, 2012). Differential expressions of phosphate starvation-responsive genes under abiotic and biotic stress likely involved structural alteration in the roots of plants treated with NPs.

Wounding

Damaged plant tissues are potential sites of bacterial or viral infection and may decrease both quality and yields. After mechanical damage, many plants develop an enhanced resistance to further pathogen attack in which ROS are synthesized within a few minutes of wounding. Plant response to wounding involves cell-wall modifications, namely, the deposition of lignin and suberin, and an increase in cell-wall integrity (Reymond *et al.*, 2000). The only available study regarding molecular defense motivated by NPs under wounding stress is García-Sánchez *et al.* (2015), which discusses treatment of *A. thaliana* with three types of NPs under five biotic and abiotic stress inducers. In contrast to other biotic and abiotic stresses, none of the 351 NPs-responsive genes are responsive to wounding stress in the early-stage response of *A. thaliana*. The absence of wounding-responsive genes might be due to the integrated defense mechanisms against both wounding and pathogen infection (Savatin *et al.*, 2014). Even so, SAR is stimulated by certain pathogens or by mechanical damage, like wounding by herbivorous insects. SAR enhances plant defense by increasing the thickness of the cell wall and by altering other physiological processes (Dempsey and Klessig, 2013).

Conclusion and Future Perspective

Understanding the molecular defense mechanisms associated with the alleviation role of NPs is helpful in protecting plants from stress. There are a limited number of studies that investigate the molecular mechanisms triggered by NPs against abiotic and biotic stress. Despite their relative scarcity, these studies do provide an illustration of how NPs activate molecular defense against stress. The available reports also demonstrate the ability of NPs to increase plant tolerance to environmental stress by decreasing the severity of injury and increasing the capacity of defense systems. This paper has reviewed all the available research results and, in doing so, it has documented the common molecular defense mechanisms triggered by NPs in plants subjected to major stressors.

The studies discussed in this review focus on the overlap between stress-responsive genes and NPs-responsive genes. Thus, these studies target only the gene networks that are involved in NPs-based alleviation. Furthermore, some of the selected studies compare the molecular responses of tolerant and sensitive plant cultivars to NPs under the same stress conditions. These comparisons reveal a mutual response between plants treated with NPs and tolerant cultivars, suggesting that tolerant cultivars tolerate stress through the same defense mechanisms promoted by NPs. Altogether, the NPs-based alleviation mechanisms investigated here appear to involve the regulation of genes responsible of protein synthesis, cell division/organization, amino acid metabolism, antioxidant activity, ROS scavenging, root hair development, iron uptake and transport, and nutrient uptake.

Moreover, the ABA and SA hormones are shown to be regulated by NPs-based alleviation. The ABA-dependent pathway is found to be activated in response to NPs under salt stress, whereas SA signalling is activated by NPs for combating plant disease. Genes involved in other types of hormonal signalling are repressed by NPs under salt stress. Furthermore, NPs-based alleviation of antibiotic stress appears to involve the same molecular mechanisms used to combat heavy metals contamination. Genes involved in ion uptake and transport are regulated by NPs under antibiotic and heavy metal stresses. These genes are responsible for transporting toxic agents from the cytosol into the vacuole. Additionally, activating bioenergy and metabolism genes are observed as common responses to NPs under several types of stress. For instance, switching from aerobic respiration to anaerobic fermentation appears to be a common mechanism under flooding and cold stress.

The molecular defense mechanisms promoted by NPs are involved regulating the expression of stress-responsive genes by transcriptional repression. For instance, repression of antioxidant enzyme genes by NPs is also common under several stresses. Genes related to root hair development and fermentation are also repressed by NPs under different stress sources. These regulation patterns of NPs indicate that the

alleviation role of NPs is essentially based on transcriptional repression, following a common pattern regardless of the type or size of the NPs. Repression of gene expression is an important mechanism in plants' immune systems' management of stress. It is also an essential mechanism for establishing intricate spatio-temporal patterns of gene expression during plant development (Hanna-Rose and Hansen, 1996). Besides transcriptional repression, other gene-regulation strategies are shown to involve in biotic and abiotic stress responses in plants. Post-transcriptional regulation of gene expression by alternative splicing (Carvalho *et al.*, 2016), microRNA and small interfering RNAs molecules (Khraiwesh *et al.*, 2012) appears to be stimulated during stress. The interaction of NPs and plant response to stress appears to involve regulation of gene expression at mRNA level via microRNA molecules (Burklew *et al.*, 2012; Frazier *et al.*, 2014). Moreover, plant stress responses involve also an epigenetic regulation of gene expression (Boyko and Kovalchuk, 2008; Chinnusamy and Zhu, 2009). Epigenetics involves regulation of gene expression through DNA methylation or histone modification without alteration in DNA sequence (Berger, 2007). More recently, the epigenetic modifications and mRNA silencing are found to contribute together by regulating gene expression in response to the stress (Pontes *et al.*, 2009; Molnar *et al.*, 2010; Wu *et al.*, 2010). However, the mitigation role of NPs through regulation at mRNA or epigenome levels is still not fully understood. Thus, the stimulation of epigenetic mechanisms and post-transcriptional regulation by NPs should be given more attention by research in order to better comprehend alleviation mechanisms.

The alleviation role of NPs is limited to specific dosages of NPs, while lower or higher concentrations of NPs are known as a source of abiotic stress (Jha and Pudake, 2016; Siddiqi and Husen, 2017). This mitigation role of specific dosage of NPs to a wide range of stress might due to the positive effect of stressors combination in enhancement of plant tolerance. Many reports confirm the suggestion that the simultaneous combination of an abiotic stress with a biotic stress can enhance host plant susceptibility to pathogenic organisms and insects (Pandey *et al.*, 2017). Furthermore, recent studies reveal that plant tolerance to combinations of two or more stressors is unique and distinct from the plant's response to each of the different stresses separately (Suzuki *et al.*, 2014). However, improving plant tolerance by a combination of abiotic and biotic stresses needs further studies in order to explore related molecular defense mechanisms.

Currently, most of recent studies have focused on the alleviation role on NPs at the morphological, anatomical and physiological levels. However, limited studies have investigated transcriptome and proteome changes in response to NPs under stress. There are complex molecular mechanisms beyond the observed effect which cannot be predicted through morphological and physiological

measurement. Likewise, understanding the intricate mechanisms concerned with the mitigation processes through these conventional methods is unattainable, due to the complexity of the biochemical pathways that involved in the stress response. Gene networks that involved in stress alleviation are not understood as many genes that participate in plant defense mechanisms need to be identified. High-throughput transcriptomic and proteomic technologies can provide valuable data for expression profiling during various biological processes (Kumar *et al.*, 2016). Along with bioinformatic tools, these techniques can help in identifying novel genes and revealing the complicated gene networks related to NPs alleviation role under stress. Uncovering these molecular mechanisms must be considered as an important goal for agricultural and biological studies. This review has shed light on the molecular mechanisms triggered by NPs under different types of abiotic and biotic stress. However, further transcriptomic and proteomic studies are required in order to develop a deeper understanding of gene regulation during plant response to stress.

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