COMPARATIVE ANALYSIS OF ZINC OXIDE NANOPARTICLES INDUCED TRANSCRIPTOMIC RESPONSES IN ARABIDOPSIS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Azita Alasvand Zarasvand

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Genomics and Bioinformatics

July 2019

Fargo, North Dakota

North Dakota State University
Graduate School

Title

COMPARATIVE ANALYSIS OF ZINC OXIDE NANOPARTICLES INDUCED TRANSCRIPTOMIC RESPONSES IN ARABIDOPSIS

By

Azita Alasvand Zarasvand

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Robert Brueggeman
Chair

Dr. Phillip E. McClean

Dr. Achintya N. Bezbaruah

Approved:

July 30, 2019
Date

Dr. Philip McClean
Department Chair

ABSTRACT

The impact that zinc oxide nanoparticles (ZnONP) have on plant physiological responses was evaluated by comparing gene expression changes after *Arabidopsis thaliana* plants were exposed to ZnONP, in comparison with ionic Zn^{2+} (ZnSO4) and non-treated controls. After treatment with ZnONP (concentration10 μg L−1), ionic Zn^{2+} (applied as ZnSO4 at a concentration of 19.7 μg/ L −1), expression analyses via RNA sequencing revealed that 369 genes were down regulated and 249 were upregulated ($p <$ [FDR] 0.05, expression difference < 3). The downregulated genes in ZnONP treated seedlings compared to the Zn^{+2} ion and untreated controls were mainly abiotic stress (oxidative stress, low temperature) and biotic stress such as defense responsive genes based on the gene ontology analysis. The upregulated genes in response to ZnONP treated plants compared to the Zn^{+2} ion control plants were mainly photosynthesis, light harvesting complex, and hormone responsive genes such as abscisic acid.

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Robert S. Brueggeman of the Plant Pathology Department at North Dakota State University for the continuous support of my Master study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time.

I would also like to thank the experts who were involved in the validation survey for this research project: Prof. Phillip E. McClean Director of the Genomics & Bioinformatics Program and Prof. Achintya N. Bezbaruah Without their passionate participation and input, the validation survey could not have been successfully conducted.

TABLE OF CONTENTS

LIST OF FIGURES

1. INTRODUCTION

1.1. Literature review

Nanotechnology is a trans disciplinary field of science, but at its core is the engineering of materials that are 1 to 100 nanometers in size (Salata, 2004). The total global investment in nanotechnologies was more than $4 billion in 2005 and it is being developed at several levels including materials, devices, and systems (Roco, 2005). The major biological focus of nanotechnology is human medicine. However, nanoparticles (NPs) are being engineered to address issues in agriculture including biotic and abiotic stress management, as fertilizers and for nutrient fortification. Thus, nanotechnology has the potential to revolutionize agricultural production and the food industry through crop disease management, adjustment of water and nutrient use efficiency in crops, and by increasing the plants ability to absorb nutrients or pesticides (Prasad et al., 2014). Furthermore, nano biotechnology, such as sensor development, will increase our knowledge of the molecular biology and physiology of various crop plants ultimately enhancing food production. Nano based biosensor development will also allow for improvement of environmental conditions monitoring which will aid in the development of disease forecasting models and to monitor soil and plant conditions to better understand how abiotic stresses affect crop production. This real time monitoring of environmental and *in planta* conditions will also have application in phenomics research allowing for more reliable phenotyping that can ultimately aid in plant breeding and selection of the best genotypes for biotic and abiotic stress resistances.

There is a myriad of nanoparticles being used or developed for agricultural uses including zinc oxide, iron oxide, copper oxide, aluminum oxide, titanium dioxide, silver, gold, silica, carbon nanotubes, and graphene (Sabir *et al.*, 2014; Jain *et al.*, 2018). Engineered nanomaterials

have been divided into four categories, (i) carbon-based materials, (ii) metal-based materials, (iii) nanosized polymers; and (iv) integrated nanoparticles with other bulk scale materials (Stampoulis et al., 2009). The explosion of this technology has been made possible because of the desirable properties of nanoparticles, which include a high surface area to volume ratio that increases surface reactivity. The small size, structure, and surface characteristics of nanoparticles, which are not observed for their bulk particle counterparts, give them unique physicochemical and biochemical properties. For instance, particles with dimensions less than 5 nm have unique electronic states and catalytic reactivates that differ from corresponding bulk scale materials (Kim et al., 2008). Moreover, insoluble substances have more solubility when the particle size is less than 100 nm. Thus, nanoparticles have a greater surface area compared to the particles with equivalent mass, which cause the proportion of atoms on the surface to be greater than the interior ofthe structure, providing high surface reactivity. As an example, nanofertilizers at lower dosages can have more desirable effects than traditional fertilizers at higher dosages (Auffan et al., 2009).

Currently, nanomaterials are widely applied to the marketplace in electronics, optics, food packaging, textiles, medical devices, cosmetics, water treatment technology, fuel cells, catalysts, biosensors, and components for environmental remediation (Kim et al., 2008). Silver (Ag) nanoparticles also have antimicrobial characteristics, which make them applicable in a range of products, such as textiles, bandages, air filters, and vacuum cleaners. Carbon-based nanomaterials are used in plastics, catalysts, battery cell electrodes, water purification systems, and orthopedic implants (wang, 2008). Thus, this wide range of applications suggests that these materials will make their way into natural ecosystems where they can interact with diverse natural plant populations.

Both positive and negative effect of nanoparticles on plants have been previously reported. For example, spinach (*Spinacia oleracea*) exposed to nano-TiO2 showed increased seed germination and had enhanced plant vigor with improved growth, chlorophyll formation and rubisco activity. Nano-TiO2 was also shown to enhance the rate of photosynthesis in spinach (El-Temsah., 2014). The application of SiO2 nanoparticles on soybean (*Glycine max*) enhanced nitrate reductase activities in root and leaves, and could increase their abilities to take up water, which improved seed germination. It also stimulated antioxidant production leading to increased resistance to pathogens (14). Kalteh et al (2014) examined the effectiveness of nano-SiO2 on Basil (*Ocimum basilicum*) under salinity stress. They showed that it increased the chlorophyll content, proline accumulation and the activity of antioxidant enzymes that also provided greater biotic stress tolerance to the plants (Kalteh et al., 2014). Arora et al. (2012) showed that the application of gold nanoparticles on *brown mustard (Brassica juncea) also* had positive effects on plant development that improved growth and yield by enhanced leaf numbers per plant, greater leaf area, taller plants, and increased chlorophyll and sugar content (Arora et al. 2012).

The use of the model plant Arabidopsis allowed Kumar et al. 2013 to begin elucidating the molecular mechanisms underlying the positive effects of AuNPs on plants. They exposed the model plant *Arabidopsis thaliana* to AuNPs and found that the expression of microRNAs (miRNAs) was significantly decreased in the plants exposed to NPs (Szymanski et al., 2003). As miRNA research is beginning to show that they are major regulatory molecules in plants that impact the expression levels of their target genes, these findings are quite important and fascinating (Feng et al, 2010). The Overexpression of miRNAs have been shown to decrease the activity of antioxidant enzymes, prevent seed germination, inhibit seedling growth and root elongation (Feng et al, 2010). Therefore, the decrease in the expression level of miRNAs was

hypothesized to lead to enhanced seed germination, seedling growth and root elongation compared to the control plants.

Previous research had also considered the effect of titanium dioxide nano particles (TiO2NPs) on plant development. Mahmoodzadeh et al. (2013) showed that TiO2NPs increased seed germination and plumule growth of canola (*Brassica napus*) seedlings (Mahmoodzadeh et al., 2013). Jaberzadeh et al. (2013) found that TiO2NPs promoted agronomic traits in wheat (*Triticum aestivum*) such as gluten and starch content. Since, water deficit stress reduced the plant growth and yield components, the application of TiO2NPs is recommended under water stress condition (Jaberzadeh et al., 2013).

It was also determined that TiO2NPs enhanced light absorbance accelerating transport and conversion of light energy, while increasing the time that the chloroplasts were efficient at photosynthesis which was interpreted as preventing the chloroplasts from aging. This may be possible due to the NPs being able to promote the activities of superoxide dismutase, catalase, and peroxidases which can reduce the accumulation of reactive oxygen free radicals that helps to keep the structure of chloroplast membranes stable under light induced conditions (Hong et al., 2005; Yang et al., 2006).

Other positive impacts on plant development and growth discovered across a range of nanoparticles and their interactions with diverse plant species has led to the exploration of physical and chemical characteristics of different classes of NPs for the intelligent development of nanomaterials with directed or specific functions. For example, a major NP that is being explored as a carrier of biological materials or reactive chemicals are carbon nanotubes (CNTs) because of their unique mechanical, electrical, thermal and chemical characteristics. They can penetrate into the cell wall and membrane of cells which helps them to carry chemicals into cells.

The single-walled-CNTs have been shown to have the capacity to transports DNA into plant cells (Srinivasanet al.,2010). It also has been found that multi-walled-CNTs (MWCNTs) can influence seed germination and plant development possibly via increased uptake of Ca and Fe nutrients (Villagarcia et al., 2012). Lahiani et al. 2013 showed that the MWCNTs increased the expression of genes encoding water channel proteins in soybean, corn, and barley seeds that lead to positive impacts of MWCNTs on germination and growth of seedling (Lahiani et al., 2013). Wang et al. (2012) evaluated the effects of oxidized MWCNTs on the development and physiology of wheat plants. Transmission electron microscopy images showed that MWCNTs entered the cell wall and the cytoplasm after being absorbed by roots. They observed that MWCNTs promoted root cell elongation and enhanced dehydrogenase activity, which was hypothesized to facilitate root growth and the accumulation of significantly more biomass (Wang et al., 2012). However, it had also been reported that MWCNTs did not show a positive impact on seed germination in some plants, even at high concentrations (Husen et al., 2014).

Krishnaraj et al. 2012 considered the impact of biologically synthesized AgNPs on hydroponically grown waterhyssop (*Bacopa monnieri*), an herb that is commonly used to enhance brain cognition. It was observed that AgNPs did not affect seed germination, however, it did reduce protein and carbohydrate content and altered metabolite content by enhancing Phenol content. The *B. monnieri*-AgNP interaction resulted in the induction of reactive oxygen species (ROS) resulting in oxidative damage to the plant cells, consequently, enhancing catalase and peroxidase activity (Krishnaraj et al., 2012). This response is commonly associated with plants exposed to biotic stresses or pathogen challenge (Lattanzio et al., 2006). Gruyer et al. 2013 also examined the outcome of AgNP-plant interactions using barley and lettuce. They found that AgNPs could have positive or negative impacts on plants of different species. It was observed

that root length was enhanced in the monocot model plant barley but was decreased in the dicot plant lettuce (Gruyer., 2013).

Silver nanoparticles are also applied as antibacterial nanomaterials, and different AgNP morphologies have been shown to have different levels of antimicrobial activity. To determine if these different AgNP morphologies have differential effects on plants Syu et al. (2014) investigated the physiological responses and gene expression of *Arabidopsis* after interaction with three different morphologies of AgNPs. The spherical AgNPs with the smallest size showed the highest levels of antibacterial activity and the highest levels of induced expression of Cu/Zn superoxide dismutase (SOD). The Cu/Zn SOD is a chloroplast localized SOD that is involved in balancing reactive oxygen species (ROS) especially when a plant is under biotic stresses. The ROS accumulation response is required to defend against the threat, yet the plant must deal with the elevated ROS levels to maintain its own cellular homeostasis and survival. They also showed that the decahedral AgNPs showed the lowest Cu/Zn SOD expression response in *Arabidopsis* but induced the highest level of root growth promotion (RGP). Spherical AgNPs did not affect RGP but induced the highest levels of anthocyanin accumulation in *Arabidopsis* seedlings, which is also a biotic and abiotic stress response. Furthermore, the three different types of AgNPs impacted the expression of the cell-division-cycle kinase 2, protochlorophyllide oxidoreductase, and fructose-1,6 bisphosphate aldolase genes which impact plant development, photosynthesis, gluconeogenesis and glycolysis. They concluded that AgNPs affect many important processes in plant development and responses to environmental stimuli like root growth promotion and ROS accumulation by inducing the expression of genes involved in cellular events, such as cell proliferation, metabolism, and hormone signaling pathways (Syu et al., 2013). Yet, the morphology of the NP played a role in how both microbes and plants responded differentially to

the AgNPs suggesting that the physical properties of the NPs are important for how they act as plant elicitors or are perceived by plants.

One of the most used NPs in agriculture are the zinc oxide NPs (ZnONPs) that are currently being tested for their efficacy as pesticides, fungicides, herbicides, fertilizers, nanobiosensors, and for water purification. Zinc is also an essential micronutrient for humans, animals and plants, thus, ZnONPs was the class of nanoparticles used in this study. Many studies have shown that ZnONPs affect plant growth and development. Zinc (Zn) is the second most abundant transition metal in organisms after iron and function in all six enzyme classes including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Plants take up Zn as the cation Zn2+. A series of enzymes contain Zn that play an important role as a functional, structural or a regulatory co-factor. Zinc deficiency leads to some of the physiological reprogramming that disturb normal enzyme activity. Therefore, it can affect photosynthesis with a decrease in activity of key photosynthetic enzymes (Prasad et al., 2012). Zinc is also necessary for chlorophyll production, pollen function, fertilization and germination (Kaya et al., 2002). Sedghi et al. 2013 showed that the application of 1 g/L ZnONPs on soybean could increase germination percentage under drought conditions (Sedghi et al., 2013). Ramesh et al, 2014 reported that ZnONPs did not show any adverse effects on the percentage of seed germination, shoot-root growth and cell division when applied to *Triticum aestivum* (wheat). However, chlorophyll and protein content were promoted after treating with ZnONPs (Ramesh et al., 2014). A decrease in mitotic index and increase in chromosomal abnormalities were reported at higher concentrations (40 ug/ml) of ZnONPs applied to onion. However, in lower concentration 20µg/ml maximum percentage of seed germination and seeding growth was reported at 96.52% compared to untreated seeds which had 94.28 % seed germination (Raskar et

al., 2014). The application of ZnONPs on alfalfa (*Medicago sativa*), cucumber (*Cucumis sativus*), and tomato (*Solanum lycopersicum*) seedlings revealed that only germination in cucumber was increased by 10 % at 1600 mg/L but on alfalfa and tomato germinations were decreased by 40 and 20 %, respectively (de la Rosa., 2013). It also was reported that foliar application of ZnONPs improved plant biomass, shoot length, root length, root area, chlorophyll content, total soluble leaf protein, rhizospheric microbial population, acid phosphatase, alkaline phosphatase and phytase activity in clusterbean (Raliya et al., 2013). Application of 100 mg/L ZnONPs on banana enhanced the percentage of somatic embryogenesis, shoot growth, and root growth. It also led to an increased activation of the enzyme superoxide dismutase, catalase, and peroxidase that can increase plant resistance to biotic stresses or pathogen challenge (Helaly et al., 2014). The effect of nano-scale ZnO as a seed coating on maize (*Zea mays* L.), soybean (*Glycine max* L.), pigeon pea (*Cajanas cajan* L.) and ladies finger (*Abelmoschus esculentus* L.) indicated that the germination percentage was enhanced to 93-100% compared to uncoated seeds which was ~80%. Moreover, it enhanced the auxin indole-3-acetic acid (IAA) production in plant roots that influence the improvement of root growth (Adhikari et al., 2016). Subbaiah et al., 2016 showed that ZnO-nanoparticulates could increase the germination percentage and seedling vigor in maize (*Zea mays L*). Also, it promoted the growth, yield, and zinc content of maize grains that is useful for human health (Subbaiah et al., 2016). It was also reported that ZnONPs at 1000 µg/mL on maize resulted in 'tunneling-like' effects of the primary root tip but did not cause significant effect on seed germination (Pokhrel et al., 2013). Foliar application of ZnONPs at a 2 g/L concentration increased the activation of superoxide dismutase (SOD), shoot dry weight and had a positive impact on biomass production of sunflower under salinity conditions (Torabian et al., 2016). The application of 100 mg/L ZnONPs to the roots of

Arabidopsis thaliana induced the up and downregulation of 660 and 826 genes, respectively. The up-regulated genes are involved in both abiotic (oxidative, salt, water deprivation) and biotic (wounding and defense to pathogens) stress responses. The down-regulated genes were implicated in cell organization and biogenesis, including translation, nucleosome assembly and microtubule-based processes (Landa et al., 2012).

Almeelbi and Bezbaruah, 2014 found that the nanoscale zero-valent iron (NZVI) is useful for increased bioavailability of phosphate for the plants. They showed application of NZVI enhanced spinach biomass and iron content in roots, leaves, and stems 11-21x in spinach after exposure (Almeelbi and Bezbaruah., 2014). Foliar application of IONPs had positive impact on root elongation and photosynthetic rate due to enhancement of stomatal opening in Glycine max (Alidoust et al., 2013).

Application of 20 mg/L g-Fe2O3 NPs showed positive effect on root elongation increased germination index and vigor index. δ-Fe2O3 nanoparticles (IONPs) caused elongation of shoot and root growth compared to the bulk counterpart (IOBKs-Cit) in rice. It was concluded that a decrease in the pH of rhizoplane soils was caused by higher accumulations of Fe on the root surfaces of bulk treatments. Therefore, it affected shoot and root growth reduction (Alidoust et al., 2014). Exposure of *Arabidopsis thaliana* to NZVI increased the overexpression of AHA2 that made high plasma membrane H+-ATPase activity. The activation of H+-ATPase promoted stomatal opening on leaves and led to enhance assimilation of CO2. This trend could be harnessed as a solution to increase the resistance of plant to drought stress (Kim et al., 2014).

Although many positive effects of NPs on plant development and physiology have been reported there are also negative outcomes of these interactions as well. Yang and Watts (2005) showed that uncoated nanoscale Al2O3 inhibited root elongation of corn (*Zea mays*), cucumber

(*Cucumis sativus*), soybean (*Glycine max*), cabbage (*Brassica oleracea*), and carrot (*Daucus carota*) (Yang and Watts., 2005). The application of 50 and 100 mg/L g-Fe2O3 NPs had adverse effect on hydroponically grown corn (Li et al., 2016).

There have also been adverse effects of the application of ZnONPs reported; the NP used in this study. For example, the exposure of 0.4 g/L ZnONPs on *Allium cepa* caused the loss of membrane integrity, increased chromosome aberrations, micronucleus formation, and DNA strand breaks. In addition, increase intracellular ROS production, lipid peroxidation, and activities of antioxidant enzymes was observed in *Vicia faba* and *Nicotiana tabacum* (Ghosh et al., 2016). Lin and Xing (2007) showed that the application of ZnONPs inhibited germination and root growth of radish (*Raphanus raphanistrum*), rapeseed (*Brassica napus*), ryegrass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn, and cucumber (Lin and Xing et al., 2007). The application of ZnONPs on corn and cucumber showed negative impacts on the root length of corn and cucumber but did not exhibit any changes in seed germination (Zhang et al., 2015). The interaction of 500 mg/kg of ZnONP in the soil with pea led to a decrease of chlorophyll content in leaves (Mukherjee et al., 2014). The negative impact of ZnONPs has also been observed in soybean (*Glycine max*) resulting in damage during developmental stages and reproduction. García-Gómez et al., 2014 reported that ZnO-NPs had adverse effects on emergence, root elongation, and shoot growth in radish (García-Gómez et al., 2014). Interestingly, Watson et al., 2015 showed that the soil properties affect the influence of nanoparticles on plant growth. They showed ZnONP in acidic soil prevented the elongation of roots of wheat but phytotoxicity of ZnONP was not observed in calcareous alkaline soil (Watson et al., 2015). These studies exemplify the possible negative impacts of nanoparticles on a diversity of crop plant species, but

there are also environmental factors which can influence the effects, positive or negative, that these interactions have on plants of diverse species.

1.2. Plant disease immunity receptors

Due to the impact of plant diseases on food security it is an important global issue that has been amplified in recent years due to climate change issues and the effects it has on plant disease spectra. The warmer and more humid climates in some regions of the world are resulting in more and newly emerging fungal diseases in crops. This has resulted in the need for increased focus on improving approaches to crop protection. One of the major strategies to control disease is breeding crop plants for resistance. This approach is based on the gene-for-gene' relationships between host resistance genes and pathogen virulence factors. Plant resistance genes elicit the expression of components of the plant immune system that enable plants recognize and respond to specific pathogens. Plants apply two approaches to recognize pathogens (Chisholm et al., 2006; Jones et al., 2006). Diverse pathogen species carry conserved microbial elicitors known as pathogen associated molecular patterns (PAMPs) that are detected by receptor proteins known as pattern recognition receptors (PRRs). Activation of PRRs elicit the early non-host specific resistance mechanisms known as PAMP-triggered immunity (PTI). These early active immunity responses in plants are effective against diverse pathogen species and represent the first line of active defense in plants against potential microbial pathogens (Boller et al. 2009).

PRRs are classified in two classes including transmembrane receptor kinases and transmembrane receptor-like proteins (Zipfel et al., 2008). The flg22 peptide, conserved PAMP, binds the PRR in *Arabidopsis thaliana* called FlAGEllIN SENSING 2 (*FLS2*) that leads to activate signaling complex. FlS2 makes a complex with BAK1 that initiate PTI signaling pathway. BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1) belongs

to family of receptor kinase (SERK) members which is also called SERK3 (Schulze et al., 2010). PAMPS induce production of ROS in the form of superoxide anion and hydrogen peroxide. A MAPK cascade including MEKK1-MKK4/5-MPK3/6 is the downstream PTI signaling pathway of FLS2 in *Arabidopsis* (Asai et al., 2002). The recognition of PAMPS also induce stomata closures that plays a major role in keeping the pathogens from entering the natural openings and gaining entry to the host (Lee et al., 2009; Melotto et al., 2006).

The second line of active defense that plants utilize to detect an invading pathogen is recognition of pathogen derived molecules or elicitors by intracellular receptors that mainly belong to the nucleotide binding-leucine rich repeat (NLR) class of cytosolic localized immune receptors. The NLRs are the typical disease resistance proteins that mediate a higher amplitude defense response that has been designated as effector-triggered immunity (ETI). The NLRs are classified based on their N-terminal domains that can include a predicted coiled-coil N-terminal domain (CC-NLRs) or an N-terminal domain with homology to the Toll/Interleukin-1 like receptor (TIR-NLRs). The NLRs act as intracellular immune receptor that recognize specific pathogen effector proteins. The NLR proteins detect effectors via direct binding or indirectly by recognizing the effectors manipulation or modification of host virulence target proteins (Jones et al., 2006). Plants respond to pathogen recognition by inducing a strong, yet localized form of programmed cell death known as the hypersensitive response (HR) (Coll et al., 2011). One of the best characterized effector targets in plants is the RIN4 (RPM1-interacting protein 4) protein that is involved in indirect mechanisms of resistance signaling. In the presence of *P. syringae* effectors, AvrRpm1, RIN4 is hyperphosphorylated and this modification of RIN4 by the pathogen effector is recognized by the NLR receptor RPM1 which activates ETI responses including HR which suppresses the bacterial pathogen in dead cells (Chisholm et al., 2006). It

also has been found that the NON-RACE-SPECIFIC DISEASE RESISTANCE 1 (NDR1) protein also interacts with RIN4 at the cytoplasmically localized N-terminal domain of NDR1, which activates defense signaling pathway upon infection with *P. syringae* (Day et al., 2006).

1.3. Mechanisms of nanomaterial induced physiological reprogramming

The impact of nanoparticles on cell death, genotoxicity, and the production of reactive oxygen have been showed. It has been found that exposure of nanoparticles led to the activation of a variety of signaling pathways that are involved in immune responses in mammals. For example, exposure of human lymphoid cells to metal oxide NPs led to the activation of mitogen-activated protein kinase pathway and expression of genes that play a role immune response (Simon-Vazquez et al., 2016). There are also examples in the literature of nanoparticles eliciting defense response pathways in plants.

Based on the results of many plant nanoparticle interaction studies to date, it can be posited that the outcome of novel nanoparticle plant interactions regarding plant physiological changes and their outcomes on development and the subsequent effect on plant interactions with biotic and abiotic stresses are hard to predict. Thus, the congruent and conflicting results on the impacts of NPs on plant development shows that the outcome of these interactions can vary depending on plant growth stage, the nature or class of nanoparticle, and plant species. Thus, in a monoculture agriculture system research can predict the outcome of a class of nanoparticles on a specific crop species but the result of application to the field in the peripheral natural ecosystem including the subterranean soil microbiota and rhizospere cannot be realized. The objective of this study is to consider the effect of ZnONPs on *Arabidopsis thaliana* and the subsequent induced physiological reprogramming that include the innate immunity system.

1.4. Plant absorption and uptake of nanoparticles

The plant uptake of nanoparticles is affected by many factors such as the nature of the nanoparticle plant physiology and the interaction of the nanomaterials with the environment. Nanoparticle size is of the main restrictions to penetrate plant tissues, and it has been reported that the dimensions that plants can absorb it, are usually with 40–50 nm. Moreover, the type of nanoparticle and its chemical composition is another trait that influence its uptake by plant (Pérez-de-Luque, 2017). Functionalization and coating of the nanomaterial surface can affect properties for its absorption by the plant (Judy, 2017). The plant physiology that is different in plant species also can influence the absorption of nanomaterials by plants. The interaction of nanoparticles with other components of the environment can affect their traits to be assimilated by plants (Pérez-de-Luque, 2017). For instance, humic acids and other organic matter in the soil can improve stability and bioavailability of nanoparticles while salt ions might induce precipitation and make a contrary effect (Navarro, 2008). There are two ways that nanoparticles can penetrate the plant: the apoplast and the symplast. Apoplastic transport is outside the plasma membrane through the extracellular spaces, cell walls of adjacent cells and xylem vessels (Sattelmacher, 2001), while symplastic transport involves movement of substances between the cytoplasm of adjacent cells through plasmodesmata (Roberts *et al.*, 2003).

2. MATERIAL AND METHODS

2.1. Arabidopsis thaliana growth conditions

Arabidopsis thaliana ecotype Col-E1 seed were sterilized with 70% ethanol for 5 min, then transferred to a 20% Clorox solution for 10 min, then rinsed with water 5 times and placed in a refrigerator at 4 °C for 3 days. The sterilized *A. thaliana* seeds were grown on standard Murashige and Skoog medium (1/2 MS; Duchefa Biochemie) containing 1% sucrose, 0.05% MES, and 3g/ L phytagel. The treatments consisted of 10 µg/ L ZnONPs, ionic Zn2+ applied as ZnSO4 at a concentration of 19.7 µg/ L) and a no Zn control. The ZnONPs were added at a final concentration of 10 µg /L in the prepared MS media and sonicated for 5 min. The ZnSO4 was added to the MS media to a final concentration of 19.7 µg /L and mixed vigorously. Prepared MS medium with no zinc control was used as the no Zn controls. 70 ml of each treatment was poured into three Magenta Boxes representing three replications. Nine sterilized *Arabidopsis thaliana* ecotype Col-E1 seeds were evenly placed, three in each row and column, on the surface of the solidified media in each magenta box. The seedlings were grown under 16 h/8 h light/dark condition in a growth chamber at 22 °C, with 30% humidity. Three individual plants from each replicate per treatment at the rosette stage were harvested and immediately frozen in liquid nitrogen and placed in a -80 ^{o}C freezer until isolation of RNA was performed for the RNA-seq.

Fig. 1. *Arabidopsis thaliana* ecotype Col-E1 seedlings at the rosette stage; (A) Treatment with ZnONP. (B) Treatment with $ZnSO_4$. (C) Untreated Plants

Plants collected and stored at -80 ^{0}C

RNA Extraction

RNA Seq (Ion S5)

Analyses

Fig. 2. Plants collected and stored at -80 ^{0}C

2.2. mRNA extraction, RNA seq library preparation and RNAseq of *Arabidopsis* plants

Three individual plants from each replicate per treatment were combined in a single tube and used for total RNA extraction. The total RNA was extracted from the frozen samples using the RNeasy mini kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions.

RNA concentrations were measured using the Qubit® Broad Range RNA kit on a Qubit® 2.0 fluorimeter (ThermoFisher Scientific). The quality of RNA samples was analyzed by loading the samples on a 1% agarose gels stained with gel red (Biotium). If four crisp ribosomal RNA (rRNA) bands corresponding to the nuclear 28S and 18S rRNAs and 23S and 16S plastid rRNAs were observed, respectively, without a high molecular weight genomic DNA band were visualized on agarose gel, then the RNA was considered of sufficient quality to proceed.

For the RNAseq library preparation 1µg of total RNA was used using the NEB #E7490 (New England Biolabs, Ipswich MA) for mRNA isolation, NEB #E7771S (New England Biolabs, Ipswich MA) for first strand synthesis, NEB #E6111S (New England Biolabs, Ipswich MA) for second strand synthesis, and NEB #E6270S (New England Biolabs, Ipswich MA) for DNA library prep, according to the manufacturer's instructions. The final library was validated and quantified on the Agilent 2100 Bioanalyzer. The cDNA libraries from 9 samples, three from each treatment, were pooled into one single tube, and normalized based on the manufacturer's instruction. Each of the library pools were diluted to a concentration of 100 pm and loaded on the Ion Chef for chip prep. A single chip, Ion 540 Chip (Ion Torrent, Life Technologies, Carlsbad, CA) was used for sequencing on the Ion S5 System (Ion Torrent, Life Technologies, Carlsbad, CA). The fastq reads obtained were quality trimmed in CLC Genomics Workbench v8.0.3 (CLC bio, Aarhus, Denmark) using the default settings.

2.3. Expression analysis

The analysis pipeline for mapping reads to the reference genome, quality check, and for expression analyses was done as previously described by Sharma Poudel 2018. The high quality trimmed sequencing reads were mapped to the *Arabidopsis* Col-0 genome and gene models from Araport 11, an updated Col-0 annotation (https://www.araport.org/data/araport11) in CLC

Genomics Workbench v8.0.3 was used to obtain the *Arabidopsis* gene specific reads. Gene specific and transcript specific reads were obtained from the reference gene as well as from the gene track and mRNA tract information. This enabled reads to be aligned to both intronic and intergenic regions. Reads that were less than 90% identical for 90% of the read length and that mapped to more than 10 positions were discarded. The total reads mapped for each gene model were normalized to obtain reads per kilobase of exon model per million mapped reads (RPKM) values for each sample. In all the comparisons, the false discovery rate (FDR) corrected p-values were assayed by the exact test using the EdgeR bioconductor package in CLC genomics. Analyses were based on a threshold of 0.05 for FDR-corrected p-value and a fold change of 3.

Venn diagrams were prepared using the VENNY software to compare the differentially expressed genes (DEGs) between the treatments.

2.3.1. Gene enrichment analysis

The GO term mapping for the best *Arabidopsis* gene was downloaded from the *Arabidopsis* Col-0 genome (Araport11) (https://www.araport.org/ data/araport11) (Madden et al., 2013). GO term enrichment analysis was performed in bioconductor R package TopGO version 2.28.0 (Alexa *et al.*, 2006; Alexa e Rahnenfuhrer, 2010), and in the web-based Classification Superviewer (Provart e Zhu, 2003). In the TopGO package, Fisher's exact test was performed to calculate the significance of GO term enrichment. The cut-off for number of genes annotated for a single GO term was fixed at 5 genes with a p-value less than 0.001. In both the enrichment tools, the analyses were performed to find the significantly enriched GO terms specific to subontology categories like molecular function (MF), biological processes (BP) and cellular component (CC).

In both TopGO and Classification Superviewer, all DEGs across the treatments were subdivided into two groups, upregulated and downregulated genes, and enrichment analysis was conducted on each set separately.

3. RESULT

3.1. Expression profiling based on Venn diagrams

Of the 369 transcripts (fdr < 0.05 fc < 3) down regulated amongst comparisons of the three different treatments, 39 transcripts were down regulated in the ZnONP treated plants compared to the untreated control plants. Of these 369 down regulated genes 22 were common with the down regulated genes as compared to the untreated control plants and the Zn^{+2} ion control plants. Moreover, 8 of the downregulated genes in ZnONP treated plants were common with the down regulated genes compared to untreated control plants and the Zn^{+2} ion control plants. 80 transcripts were down regulated in Zn^{+2} ion control plants compared to untreated control plants. Interestingly, 250 genes were down regulated in ZnONP treated plants compered to Zn^{+2} ion control plants. Thus, it can be concluded that 8 (2.2%) genes were specifically downregulated due to the interaction of the *Arabidopsis* seedlings with the ZnONP.

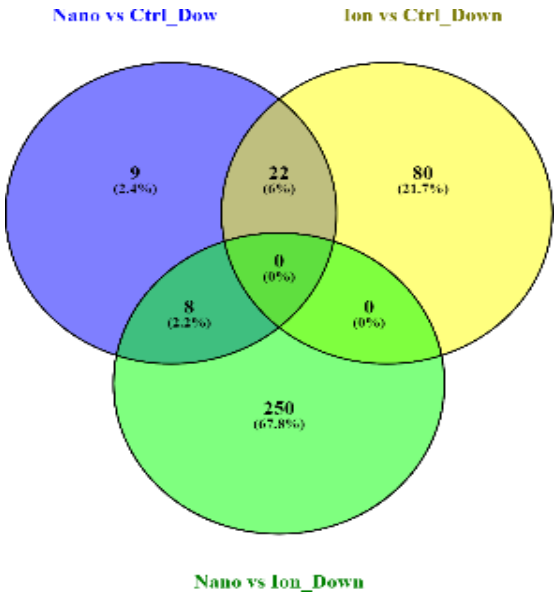

Fig. 3. Numbers of downregulated genes in response to ZnONP treated plants, Zn^{+2} ion control plants and untreated control plants

3.1.1. Transcripts downregulated in response to ZnONP treated plants compared to untreated control plants and Zn $^{+2}$ ion control plants (Appendix 1 and 6)

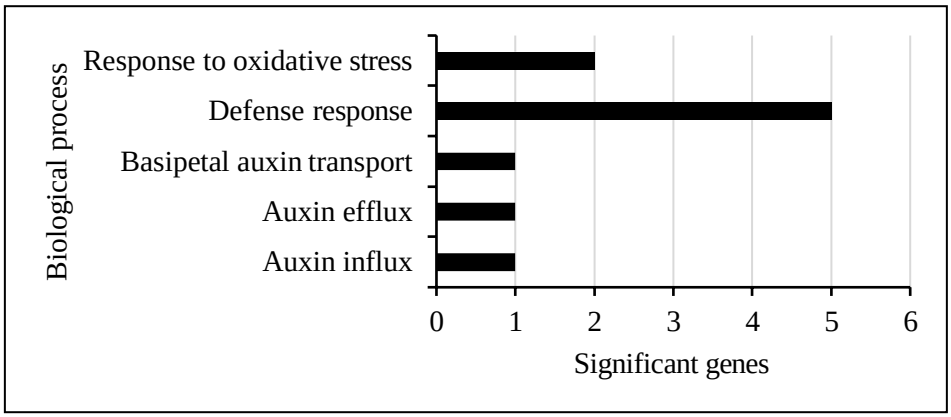

Fig. 4. Numbers of downregulated genes involved in biological process in response to the ZnONP treated plants compared to Zn $^{+2}$ ion control plants and untreated control plants

Many of the genes that were specifically downregulated in ZnONP treated *Arabidopsis* seedlings compared to the untreated control and Zn $^{+2}$ ion control plants are involved in defense responses based on the gene ontology analysis. Amongst these genes, the Cytochrome P450 gene CYP81D8 (AT4G37370), that was down-regulated 7.54-fold in the ZnONP treated plants compared to the untreated control plants and 4.62-fold compared to the Zn $^{+2}$ ion control plants. The Cytochrome P450 proteins are a group of haem-containing proteins that play a role in catalyzing different oxidative reactions (Schuler et al., 1996; Chapple et al., 1998). It has been shown that both abiotic and biotic stresses induced the expression of CYP81D8 in *Arabidopsis* (Narusaka et al., 2004).

The heavy metal transport/detoxification superfamily protein (AT5G52750) was down-regulated 10.35-fold in the ZnONP treated plants compared to the untreated control and 9.36-fold compared to the Zn $^{+2}$ ion treated control plants. The heavy metal transport/detoxification superfamily protein (AT5G52750) is a protein that has a role in heavy metal transport and heavy metal homeostasis. It has been shown to be involved in reactive oxygen signaling in response to

environmental changes such as transcriptional responses to abiotic stresses including low temperature and drought (Davletova., 2005).

The patatin-like protein 2 (AT2G26560) is a Phospholipase A 2A (PLA2A) enzyme that was down-regulated 7.54-fold in the ZnONP treated plants compared to the untreated control and 7.84-fold downregulated compared to the Zn^{+2} ion treated control plants. The PLA2A enzymes are involved in developmental and pathological processes through the production of free fatty acid (FFA) and lysophospholipids (LPL). It has been shown that PLA2A was induced in *Arabidopsis* post inoculation with the pathogen *Alternaria brassicicola* and by various abiotic stresses including low temperatures and high salinity as well as post treatment with the defense induced hormones salicylic acid and abscisic acid (Narusaka et al., 2003).

The legume lectin family protein (AT3G16530) was down-regulated 3.74-fold in the ZnONP treated plants compared to the untreated control and 4-fold downregulated compared to the Zn^{+2} ion treated control plants. AT3G16530 belongs to the lectins family of proteins which represents a superfamily of ubiquitous proteins containing at least one noncatalytic carbohydrate binding domain, called the lectin domain (Sharon et al., 2002). Arason (1996) suggested legume lectins are a family of lectins found in plants (Arason., 1996). Armijo et al., suggested that only a few lectins are involved in defense response in *Arabidopsis* (Armijo et al., 2013). Ramonell et al., showed that the lectin-like protein gene AT3G16530 was up-regulated in response to chito-octamer treatment in *Arabidopsis.* The chito-octamer treatment induces PAMP triggered immunity responses, which induces the up-regulation of genes involved in basal or non-host specific defense responses (Ramonell et al., 2005). However, it was also shown that AT3G16530 was repressed in response to drought and low temperature conditions suggesting that this gene is induced by biotic stress responses and suppressed by abiotic stresses (Wong et al., 2006).

The azelaic acid induced 1 (AZI1) protein (AT4G12470) was down-regulated 3.01-fold in the ZnONP treated plants compared to the untreated control and 4.41-fold downregulated compared to the Zn^{+2} ion treated control plants. AZI1 was classified as lipid transfer protein belonging to the subfamily of EARLI1 genes. It contains a hydrophilic proline-rich domain (PRD) and a hydrophobic eight cysteine motif (8CM). It has been shown that AZI1 and DIR1, another lipid transfer protein, function in the transfer of signals related to systemic required resistance (SAR) that are induced by azelaic acid (AZA) and glycerol-3-phosphate (Wang et al., 2016; Yu et al., 2013).

The *Arabidopsis thaliana* GLYCINE RICH PROTEIN 9, AtGRP9 (AT2G05440) was downregulated 3.53-fold in the ZnONP treated plants compared to the untreated control and 5.26-fold downregulated compared to the Zn^{+2} ion treated control plants. It belongs to the glycine-rich protein (GRP) family with high glycine content (20 to 70%) with repetitive glycine residues that form (GlyX) motifs. Tang et al. 2003 showed that NaCl and the plant hormone abscisic acid (ABA) promoted the expression level of AtGRP9. These results indicated that AtGRP9 might be involved in the salt stress response in *Arabidopsis.* In addition, they showed that AtGRP9 interacted with AtCAD5, a major cinnamyl alcohol dehydrogenase (CAD) that participates in the biosynthesis of lignin. The results demonstrated that AtGRP9 can be involved in lignin biosynthesis in response to salt stress in *Arabidopsis thaliana* (Tang et al., 2003; Chen et al., 2007).

The protein AT3G01345 which is membrane localized was downregulated 44.78-fold in the ZnONP treated plants compared to the untreated control and 120.99-fold downregulated compared to the Zn^{+2} ion treated control plants. It has been shown that AT3G01345 was downregulated in the *Arabidopsis sdg4* mutant plants. SDG4 is an *Arabidopsis* set domain

protein that modulates the expression of genes involved in the growth of pollen tubes (Cartagena et al., 2008).

These genes were downregulated in response to the ZnONP treated plants compared to the untreated control plants and compared to the Zn^{+2} ion treated control plants. Therefore, it can be concluded that down regulation of these genes is specific to the nanoparticle-*Arabidopsis* interaction.

3.1.2. Transcript downregulated in the ZnONP treated plants compared to the untreated control plants. (Appendix 2 and 7)

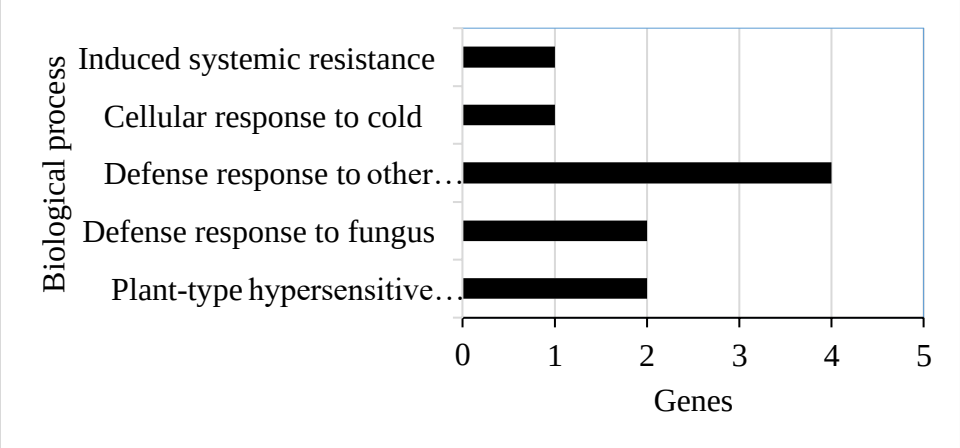

Fig. 5. Numbers of downregulated genes involved in biological process in response to the ZnONP treated plants compared to the untreated control plants.

Most of the transcripts that were down regulated in the ZnONP treated plants compared to the untreated control plants belong to the groups of genes that are involved in defense response based on the gene ontology analysis. Amongst these genes is the sulfotransferase 12 (AT2G03760), which was downregulated 3.43-fold in the ZnONP treated plants compared to the untreated control plants. It has a role in brassinosteroid metabolic processes. Sulfotransferase 12 catalyzes the transfer of a sulfate group to the hydroxyl group of brassinosteroid to produce sulfated brassionostreoid (Marsolais et al., 2007). It has been shown that brassionostreoid

mediate resistance in plants in various stress responses including low and high temperatures drought, high salt, and pathogen attack (Krishna., 2003)

AZI3 (AT4G12490) a lipid transfer protein (LTP) was downregulated 3.12-fold in ZnONP treated plants compared to untreated control plants. It has been shown that AZI1 and its closest paralog (EARLI1) are necessary for SAR. SAR signaling is regulated by salicylic acid (SA), and azelaic acid (AZA). AZA and G3P are transported by gating of the plasmodesmata (PD). PDLP1 and PDLP5 are the PD localizing proteins. AZI1 makes a complex with PDLP1 or PDLP5 for transporting of AZA (Cecchini et al., 2015).

Jacalin-related lectin 23 (JAL23) (AT2G39330) are a type of carbohydrate-binding proteins that were downregulated 3.46-fold in the ZnONP treated plants compared to untreated control plants. JAL23 is a component of the PYK10 complex. PYK10/BGLU23 is a b-glucosidase which is one of the main proteins of ER bodies (Nagano et al., 2008). The ER bodies are novel compartments derived from endoplasmic reticulum (ER) in plants. Stress conditions induce the formation ER bodies indicating that they play a role in the plant defense system (Matsushima et al., 2003).

S locus-related glycoprotein 1 (SLR1) binding pollen coat protein family (AT3G05727) is a cysteine rich protein. The SLR1 gene was downregulated 3.36-fold in the ZnONP treated plants compared to untreated control plants. The adhesion of pollen grains to the stigmatic surface is an important step during sexual reproduction. The SLR1-BP gene is expressed in pollen at late stages of development. It has been classified as a defensin-like proteins. The function of these proteins is uncharacterized (Takayama et al., 2000).

Glutathione S-transferase 6 (AT1G02930) belongs to the Glutathione S-transferases (GSTs) protein family and was downregulated 3.63-fold in the ZnONP treated plants compared

to untreated control plants. The GSTs are involved in many stress responses in plants that leads to the detoxification of xenobiotics and limits oxidative damage (Smith et al., 2004). In addition, they provide protection against oxidative stress and catalyze different metabolic reactions. GSTs catalyze the nucleophilic attack of the sulfur atom of glutathione (GSH) to the electronic center of various substrates. Glutathione mediates redox cycling between ascorbate and NADPH and has a role as a cellular storage pool of reduced thiols. During oxidative stress, rapid changes occurs in the cellular glutathione content (Kampranis et al., 2000). H_20_2 that causes oxidative injury induces the expression of *At*GSTF6 in *Arabidpsis* (Wagner et al., 2002).

The ATP binding cassette subfamily B4 (AT2G47000) gene was downregulated 7.43-fold in the ZnONP treated plants compared to the untreated control plants. It belongs to ATP-binding cassette protein subfamily B proteins. It has been suggested ABCB4 is a basic and constitutive auxin efflux transporter which plays a role in regulating cellular auxin homeostasis in the plasma membrane of *Arabidopsis* (Cho et al., 2012).

Thionin 2.1 (AT1G72260) was downregulated 10.53-fold in in the ZnONP treated plants compared to the untreated control plants. It was classified as the PR-13 family of pathogen related proteins. PR proteins play a major role in disease resistance, seed germination and enhance the plant adaptation to environmental stresses. Thionins are cysteine-rich peptides and their size are about 5 kDa. One of the main aspects of thionins is their antifungal and antibacterial effect that leads to the permeabilization of cell membranes (Sels et al., 2008). It has been shown that overexpression of the endogenous Thi2.1 thionin gene promoted resistance of *Arabidopsis thaliana* against *Fusarium oxysporum* (Epple et al., 1997).

The Pollen Ole e 1 allergen and extensin family protein (AT3G16670) was downregulated 4.00-fold in in the ZnONP treated plants compared to the untreated control

plants. They are classified as proteins with obscure features (POFs) because of their lack of defined motifs or domains. It has been shown that they were expressed in response to oxidative stress in *Arabidopsis* plants (Luhua et al., 2008).

The cell wall protein precursor (At2g20870) was downregulated 3.24-fold in the ZnONP treated plants compared to the untreated control plants. It is an unknown small protein similar to putative cell wall proteins in crucifer plants (Hu et al., 2003). It has been expressed in XERICO *Arabidopsis*, transgenic plants that conferred drought tolerance by over accumulation of ABA (Ko et al., 2006).

All the genes described were downregulated in the ZnONP treated plants compared to the untreated control plants. Therefore, it can be concluded that the downregulation of these genes is the due to the impact of the ZnONP treatment.

3.1.3. Transcripts downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. (Appendix 3 and 8)

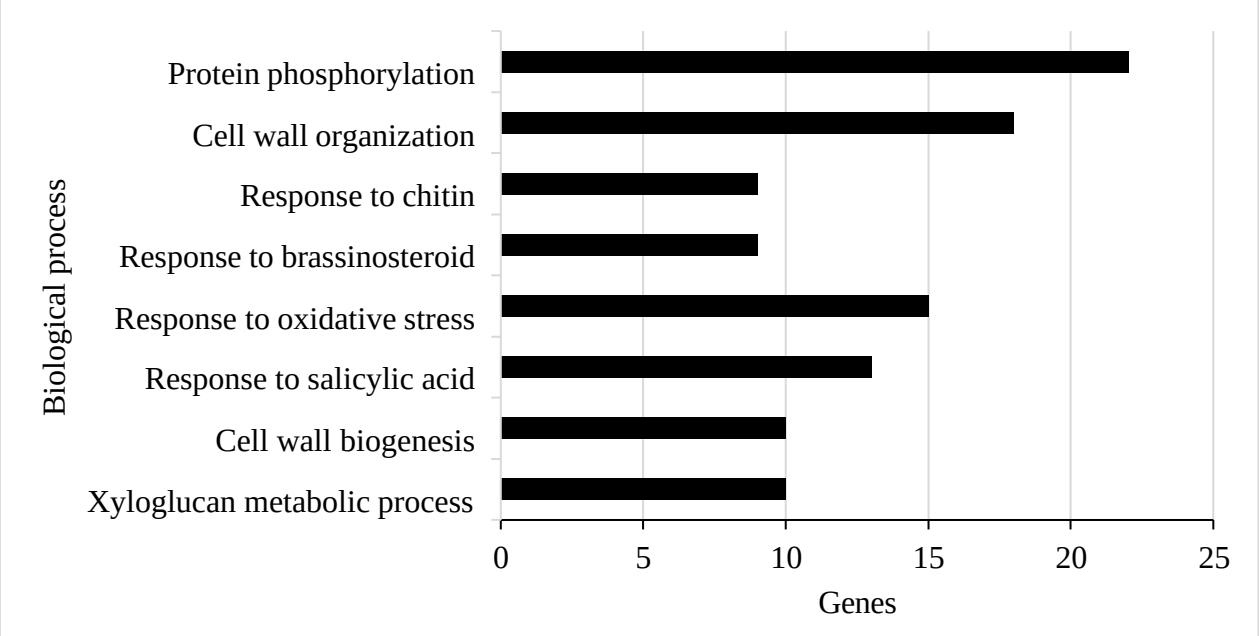

Fig. 6. Numbers of downregulated genes involved in biological process in response to the ZnONP treated plants compared to the Zn^{+2} ion treated control plants.

Genes that are involved in the stress responses represented the most abundant group of transcripts that were downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants based of the gene ontology analysis. They include genes that involved in the xyloglucan metabolite process. Xyloglucans are the one of the major hemicellulosic polymers in the cell wall of dicot plants bound to cellulose fibrils (Draeger et al., 2015). The two main mechanisms responses of plant cell walls to abiotic stress including) up regulation of xyloglucan endotransglucosylase/hydrolase (XTH) and expansin proteins, and (ii) cell wall thickening by strengthening of the secondary wall with hemicellulose (Cavalier et al., 2008). endotransglucosylase/hydrolases (XTHs) are enzymes that degrade the xyloglucans in the cell wall architecture leading to the increased cell wall extensibility and cell wall loosing (Le et al., 2015). In addition, Xyloglucan xylosyltranferases are involved in xyloglucan metabolic process in *Arabidopsis thaliana* (Hyodo et al., 2003). In this experiment, the genes that were downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants with function in the xyloglucan metabolite process were xyloglucan endotransglucosylase/hydrolase 18 (AT4G30280) downregulated 21.75-fold, xyloglucan endotransglucosylase/hydrolase 24 (AT4G30270) downregulated 9.91-fold, Xyloglucan endotransglucosylase/hydrolase family protein (AT5G57560) downregulated 8.85-fold, xyloglucan endotransglucosylase/hydrolase 25 (AT5G57550) downregulated 9.97-fold, xyloglucan endotransglucosylase/hydrolase18 (AT4G30280) downregulated 21.74-fold, xyloglucosyl transferase 33 (AT1G10550) downregulated 30.82-fold, xyloglucan endotransglucosylase/hydrolase 16 (AT3G23730) downregulated 6.22-fold, xyloglucan endotransglucosylase/hydrolase15 (AT4G14130) downregulated 10.42-fold, xyloglucan endotransglycosylase 6 (AT4G25810) downregulated 52.45-fold and xylosyltransferase 1 (AT3G62720) downregulated 4.80-fold.

The dynamic structure of plant cell walls helps to protect them against different environmental condition including biotic and abiotic stresses. Cell wall biogenesis which includes polysaccharides synthesis and the arrangement of cell wall polymers is a process that many proteins participate in (Bosch et l., 2011). The results showed the genes involving in the cell wall biogenesis were downregulated in ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They included the Cellulose-synthase-like C4 (AT3G28180), which was downregulated 3.80-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It encodes 1,4-b-glucan involved in XyG backbone biosynthesis (Cocuron et al., 2007). The cellulose synthesis is a protein complex that synthesizes the 18–36 glucan chains bonde together in a single microfibril (Liepman et al., 2010).

The leucine-rich repeat (LRR) family protein (LRX3) (AT4G13340) and LRX2 (AT1G62440) were downregulated 5.82-fold and 14.6-fold, respectively, in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They belong to the leucine-rich repeat extensins (LRXs) family which are extracellular proteins containing of an N-terminal leucine-rich repeat (LRR) domain and a C-terminal extensin domain. One of the main characteristics of this class is proteins is their hydroxyproline-rich glycoprotein (HRGP) structure (Draeger., 2015). It has been shown that the LRX gene family of *Arabidopsis* such as LRX3 and LRX2 are involve in plant development and particularly cell wall formation (Baumberger et al., 2003; Camardella et al., 2000).

Plant invertase/pectin methylesterase inhibitor (ATPME41) (AT4G02330) was downregulated 7.19-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It belongs to a pectin methylestrase super family (PMEI) that regulates the activation of PME during defense responses (Kohli et al., 2015). PMEs play a role in the

demethylesterification of pectin polymers and increase the action of endopolygalacturonases and cause cell wall loosening .It has been suggested that BRs affect the activation of PME in *Arabidopsis* under chilling stress by regulating the expression of AtPME41 (Qu et al., 2011).

Expansin-like A2 (AtEXLA2) (AT4G38400) was downregulated 3.58-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of the expansin-like A (EXLA) family in *Arabidopsis*. Expansins play a role in plant cell-wall loosening leading to cell enlargement and in various developmental processes in which cell-wall modification occurs (Cosgrove., 2000). AtEXLA2 is a positive regulator of cell elongation in the dark-grown hypocotyl of *Arabidopsis* interfering in cellulose metabolism, deposition or its organization (Boron et al., 2014).

The leucine-rich repeat receptor kinase (MIK2) (AT4G088) was downregulated 3.97-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. MIK2 is a regulator of cell wall damage responses triggered upon cellulose biosynthesis inhibition. MIK2 is essential for control of normal root growth and salt tolerance in a THE1-dependent manner. MIK2 plays a role in resistance to the fungal root pathogen *Fusarium oxysporum*. It has been suggested that MIK2 is involved in CWI sensing which impacts the regulation of various aspects of growth, as well as responses to abiotic and biotic stresses (Van et al., 2017).

Arabidopsis thaliana Wall-associated kinase 2, *AtWAK2* (AT1G21270) was downregulated 4.34-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. AtWAK2 is a receptor like protein with an extracellular domain that interacts with the cell wall and are involved in sensing of plasma membrane cell wall continuum or cellular integrity. It has been shown WAK2 binds pectin and pectin regulate inveratase transcriptional activity. Pectin activates MAPK3, a kinase that is involved in pathways regulating

developmental, stress and pathogen response which is wak2 dependent. Therefore, WAK2 is required for cell expansion and is induced by various environmental stimuli such as pathogen attack and wounding (Kohorn., 2000; Kohorn et al., 2009).

Abiotic stress is one of the biggest problems for agricultural production. Plant growth regulators have major roles in the processes of plant development. Salicylic acid (SA) is a phenolic compound that is important in signaling processes that are essential for plant development and growth. Salicylic acid is also important for dynamic responses to biotic and abiotic stress stimuli. SA can regulate plant physiological processes such as photosynthesis, antioxidant defenses under stress conditions that protect plants against abiotic stresses (Khan et al., 2015). SA also signals the production of antimicrobial compounds such as phytoalexins and pathogenesis-related (PR) proteins (Lamb et al., 1997). SA regulates plant resistance to biotrophic and hemibiotrophic pathogens. NPR1 (non-expressor of PR genes 1) is an oligomer localized at the cytosol and NPR3 and NPR4 are SA receptors. NPR1 interact with the SA receptors, NPR3 and NPR4, which results in a monomeric NPR1 that is subsequently translocated to the nucleus and interact with TGA to express PR genes (Denancé, et al., 2013). The results showed that the genes regulating SA pathway were downregulated in ZnONP treated plants compared to the Zn^{+2} ion treated control plants. Amongst these genes was the LURP-one- like protein, LURP1 (AT2G14560) which was downregulated 15.66-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of the LURP cluster and the PR1 regulon. It has been shown a 39-bp region of the LURP1 promoter regulates reporter gene expression in response to *H. parasitica* and salicylic acid. LURP1 has a single W-box and two TGA-box motifs that interacts with the W region of the PR1 promoter (Knoth et al., 2008).

The Cysteine-rich RLK (RECEPTOR-like protein kinase) CRK19 (AT4G23270), cysteine-rich RLK (RECEPTOR-like protein kinase) CRK4 (AT3G45860) and cysteine-rich RECEPTOR-like kinase (AT4G23220) (CRK14) were downregulated 3.01, 3.14 and 16.76-fold, respectively in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants . They belong to family of receptor-like protein kinases (RLKs) in *Arabidopsis*. They contain novel cysteine-rich repeats in the extracellular domains. It has been shown that salicylic acid and pathogen infection induced expression of CRK 19 and CRK 4 led to rapid cell death in *Arabidopsis* plants (Chen et al., 2004; Wrzaczek et al., 2010).

The ankyrin repeat family protein accelerated cell death 6, ACD6, (AT4G14400) was downregulated 7.23-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It contains an N-terminal ankyrin (ANK) repeat domain and a C-terminal transmembrane (TM) domain. The ANK domain contain a 33-amino acid motif that participates in protein–protein interactions. This domain is involved in various cellular processes, such as cell cycle, cytoskeleton organization, transcription and signal transduction (Lu et al., 2005). It has been shown that SA is a positive feedback loop with ACD6. Three PRRs FLS2, BAK1 and CERK1 associate with ACD6 and the expression levels of these PRRs are positively regulated by ACD6. Therefore, the major role of ACD6 is to regulate receptors. Activation of the PRRs induce the accumulation of ROS, callose deposition in the cell wall, and the generation of the signal molecule salicylic acid (SA). Therefore, it affects the response of plant to PAMPs and has a positive effect of plant defense responses (Tateda et al., 2014; Tateda et al., 2014).

Ankyrin (BDA1) (AT5G54610) was downregulated 16.8-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a protein with an N-terminal ankyrin-repeat domain and a C-terminal transmembrane domain. BDA1 is related to ACD6, a known

positive regulator of *Arabidopsis thaliana* defense responses. BDA1 is an important signaling component that acts downstream of SNC2 to regulate plant immunity. It has been suggested that SNC2 acts as a receptor or coreceptor of an unidentified bacterial PAMP signal and BDA1 functions downstream of SNC2 to activate PAMP-triggered defense responses. Activation of SNC2 leads to an increased accumulation of salicylic acid (SA) and the expression of PR (Pathogenesis- Related) genes and promote resistance to pathogens (Yang et al., 2012).

The WRKY DNA-binding protein 18, WRKY18 (AT4G31800), was downregulated 11.77-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. WRKY18 is a WRKY transcription factors that modulate pathogen-triggered cellular responses in various plant species (Rushton et al., 2010; Tsuda et al., 2015). WRKY18 and its related transcription factor WRKY40 negatively regulate resistance against *G. orontii* but positively promote resistance against *P. syringae* pv. *tomato* AvrRPS4 (Schön et al., 2013). WRKY18 and WRKY40 act as repressors of basal defense (Shen et al., 2007). It has been shown that WRKY18 target genes related to the salicylic acid (SA) pathway including ICS1, a key SA biosynthetic enzyme (Garcion et al., 2008) and EDS1, a major component required for host resistance toward different pathogens and an upstream regulator of SA signaling (Serrano et al., 2013).

The *Arabidopsis* WRKY25 (At2g30250) was downregulated 4.77-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a protein containing 394 amino acids with a molecular weight of 44.134 kD. WRKY25 was classified as a group I WRKY protein. The N-terminus and the region between the WRKY domains contains serine and/or threonine residues. It has been localized in the nucleus and recognize the TTGACC W-box sequences. It has been shown that WRKY25 is induced under stress condition and acts as a negative regulator of SA-mediated defense responses to *P. syringae*. It is consistent with the

other research that showed WRKY25 is a substrate of *Arabidopsis* MAP kinase 4, a repressor of SA-dependent defense responses (Zheng et al., 2007).

Duplicated homeodomain-like superfamily protein (AT3G11280) was downregulated 3.2-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a MYB transcription factor protein and has a DNA binding domain. It belongs to the superfamily of transcription factors that play regulatory roles in plant growth development and defense responses. It is induced in response to SA and NaCl which subsequently regulates PR gene expression (Yanhui et al., 2006; Blanco et al., 2005).

Pathogen and circadian controlled 1, PCC1 (AT3G22231), was downregulated 3.29-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a cysteine-rich transmembrane (TM) protein that belongs to the CYSTM super family (Mir et al., 2013). PCC1 was upregulated after exposure to avirulent and virulent *Pseudomonas syringae pv*. tomato, which is dependent on functional salicylic acid defense-signaling pathways (Sauerbrunn et al., 2004).

Phospholipase A 2A PATATIN-LIKE PROTEIN 2, PLA2A, which functions as a phospholipase (AT2G26560) was downregulated 4.01-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is an enzyme involves in developmental and pathological processes through the production of free fatty acid (FFA) and lysophospholipid (LPL). PLA2A was induced by various biotic and abiotic stress pathogen inoculation (*Alternaria brassicicola*), low temperature, high-salinity, abscisic acid and salicylic acid in *Arabidopsis* (Wang et al., 2016).

Fungal cell walls contain chitin a, polymer of N-acetyl-D-glucosamine, which represents a major, and best characterized fungal PAMP in plant defense responses (Boller, 1995; Stacey,

1997). It also can decrease stomata aperture opening (Lee et al., 1999; Srivastava., 2009). Some pathogens penetrate into plant tissues via open stomata (Agrios, 1997). Application of chitin promote ROS production in guard cells leading to ABA-induced stomatal closure (Klüsener et al., 2002; Veronique et al., 2002). Stomatal closure is advantageous in plant defense response against pathogen as it blocks one of the major entry points for many classes of bacterial and fungal pathogens (Klüsener, et al., 2002). The result of our experiment showed chitin responsive genes were downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control *Arabidopsis* plants. Amongst them is the AtIQM1 encode a calmodulin-binding family protein (AT4G33050) that was downregulated 3.99-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is involved in stomatal movement in *Arabidopsis*. It contains an IQ motif that is the CaM-binding domain of IQM1. It has been localized in the nucleus and cytoplasm. IQM1 promotes root growth, and stomatal opening or prevent stomatal closure. It has been shown that IQM1 negatively regulates expression of chitin signaling genes. The *iqm1* mutant showed up-regulation in chitin-responsive genes such as three WRKY transcription factor, and four mitogen-activated protein three kinase 14 (MAPKKK14) and redox responsive transcription factor 1 (RRTF1). The mutant also showed higher amount of ROS in the guard cells. Therefore, it has been concluded that the mutant has the advantage of chitin, ROS and smaller stomatal closure in defense response against pathogens (Zhou et al., 2012). However, Eulgemet al. 2004 showed AtIQM1was upregulated in the response to *Peronospora parasitica* (Downy Mildew) in *Arabidopsis.* Eulgemet al. 2004 and Zhou et al, 2012 showed that a *iqm1* mutant was not resistance against downy mildew. They also demonstrated that IQM1 does not play a role in plant response to the pathogen *Peronospora parasitica*.

The dehydration response element-binding protein 26, DREB26 (AT1G21910), was downregulated 8.05-fold the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of APETALA2 (AP2) transcription factors (TFs). These DNA binding proteins contain a AP2 domain which is 68 amino acids and called as AP2/ethylene responsive element binding factor domain (AP2/ERF) .Based on their DNA-binding regions, they were classified into subfamilies including AP2, RAV, DREB, and ERF . The AP2 transcription factors play a role in plant growth and development and in defense response. It has been found that DREB26 was upregulated in response to chitin indicating its involvement in plant immune response (Suzuki et al., 2005; Sakuma et al., 2002; Hao et al., 1996; Riechmann., et al; 1998).

The legume lectin family protein (AT3G15356) was downregulated 8.12-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It encodes a putative 30-kDa protein with a legume lectin-like domain. It is induced in response to chitin, fungal wall-derived oligosaccharide that leads to the elicitation of the plant defense response (Lyou et al., 2009).

The plant-specific GATA-type zinc finger transcription factor family protein, GATA8 (AT3G54810), was downregulated 3.33-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They are group of DNA binding proteins in eukaryotes (Reyes., 2004). The GATA DNA binding domain is a class IV zinc finger motif. GATA8 was upregulated in response to chitooctaose treatment in Arabidopsis suggesting its involvement in plant defense response (Libault., 2007).

Brassinosteroid (BR) is a Steroid hormone that promotes plant development and cell elongation. Physiological studies demonstrated that BR affects plants growth in responses to biotic and abiotic stresses. BR binds to the extracellular domain of a cell-surface receptor kinase,

BRASSINOSTEROID INSENSITIVE1 (BRI1), that initiate signal transduction cascades that regulate transcription of brassinosteriod regulation (Clouse et al., 2011; Wang., 2012). Activation of BRI1 leads to the activation of the co-receptor kinase BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) (Gou et al., 2012; Li et al., 2002). Phosphorylated BKI1 interacts with the 14-3-3 family of phosphopeptide-binding proteins to induce BR signaling (Wang et al., 2011). Then BRI1 phosphorylates plasma membrane-anchored cytoplasmic kinases including BRASSINOSTEROID-SIGNALLING KINASE1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1) that lead to the phosphorylation of BRI1-SUPPRESSOR1 (BSU1) (Tang et al., 2008). BIN2 is inactivated by BSU1 at high levels of BR (Peng et al., 2008). PROTEIN PHOSPHATASE 2A (PP2A) dephosphorylate BZR1 and BZR2 then they can move into the nucleus and bind to the promoters of their target genes to regulate gene activation or repression. (He et al., 2005). The results of our experiment showed Brassinosteroid responsive genes were downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. Amongst them is the somatic embryogenesis receptor-like kinase 4, SERK4 (AT2G13790), which was downregulated 4.17-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It belongs to the Arabidopsis SERK family, which participate in diverse signaling pathways and have functionally redundant roles (Yin et al., 2005). The SERK4 relative SERK3 interacts with BRI1 as a positive regulators of BR responses (Karlova et al., 2006; Jeong et al., 2010). BAK1 and BKK1 are required for innate immunity to hemibiotrophic and biotrophic pathogens and promote disease resistance against the hemibiotrophic bacterium *Pseudomonas syringae* and the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis* (Roux et al., 2011).

THESEUS1 (THE1) is RLK1-like kinase (At5g54380) that was downregulated 4.24-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It acts as a cell wall integrity sensor that is involves in cell elongation during plant growth and development (Hématy et al., 2007; Guo et al., 2009). THE1 interacts with GUANINE EXCHANGE FACTOR4 (GEF4) and triggers the GEF4 signaling network to regulate defense responses against the necrotrophic fungal pathogen *Botrytis cinerea*, indicating its important role in defense responses against biotic stresses (Qu et al., 2017).

The BRBT gene BZS1 (At4g39070) was downregulated 4.30-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It encodes a zinc finger transcription factor that contain two B-box domains at its N terminus without any known DNA binding domain (Khanna., et al. 2009). Expression of BZS1 was reduced under BR-deficient condition and repressed by BR. overexpressing BZS1 showed a hypersensitivity to the BR biosynthetic inhibitor brassinazole (BRZ) in transgenic *Arabidopsis* plants. In contrast, transgenic plants expressing reduced level of BZS1 had longer hypocotyls than wild type when grown on BRZ (Sun et al., 2010).

AtMEP41 (At4g02330) gene is a member of pectin methylesterases (PMEs) and was downregulated 7.18-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a demethylesterifies homogalacturonan (HG) in the apoplast. HG is the most abundant polysaccharide of pectin (Mohnen,2008; Harholt et al., 2010). Demethylesterification of pectin leads to strengthening of cell walls, because deesterified pectin form calcium bonds that makes the formation egg-box structure and pectic gels (Jarvis et al. 1984; Liners., 1989). Activation of PME was increased after inoculation with Pseudomonas *syringae pv maculicola* indicating that PMEs are involved in plant immune responses (Bethke et al., 2014). Activation of

AtMEP41 increases under chilling stress in *Arabidopsis* seedlings. It has also been observed that BR has a positive effect in promoting PME activity indicating that chilling induced PME activity depends on the BR signaling pathways (Qu et al., 2011).

The Exordium gene, EXO(AT4G08950), was downregulated 7.95-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It encodes a phosphate-responsive 1 family protein. It is a brassinosteroid (BR)-regulated gene. The EXO protein is an extracellular protein that regulates BR-induced cell expansion in leaves. EXO play a role in coordination with BR-responses to environmental and developmental signals (Schröder, et al., 2009). It has also been found that phosphate-responsive 1 was upregulated in response to abiotic stress in transgenic plants that expressed the transcriptional coactivator Multiprotein Bridging Factor 1c (MBF1c) in *Arabidopsis thaliana*. The expression of MBF1c in transgenic plants promoted the tolerance to bacterial infection, heat, and osmotic stress (Suzuki et al., 2005).

Flagellin-sensitive 2, FLS2 (AT5G46330) is a leucine-rich repeat receptor-like protein kinase that was downregulated 10.24-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It plays a role as the PRR for bacterial flagellin by identifying the epitope flg22 that triggers PTI immune responses. Upon perception of flg22, FLS2 associate with BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) which triggers the RLCKs family protein BRASSINOSTEROID-SIGNALING KINASE1 (BSK1) to be phosphorylated and disassociates from the immune complex to induce the downstream signaling pathway (Lu et al., 2010). BSK1 is a member of the RLCK subfamily and is involved in the brassinosteroid (BR) signaling pathways) and is a major component of PTI defense signaling complexes ((Tang., 2008; Shi, et al., 2013).

Reactive oxygen species (ROS) are excited forms of atmospheric oxygen (O2) that are produced in cells during aerobic metabolism. Production and accumulation of ROS can lead to extensive cell injury or death; however, they play a central role in many signaling pathways in plants involved in stress perception, photosynthesis regulation, pathogen responses, programmed cell death, hormonal action, and plant growth and development. The results showed the genes involved in the protection of plant cells from oxidative damage were downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. Amongst them was the *ATSIP2* (At3g57520) gene which was downregulated 3.7-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a putative raffinose synthase (RafS) or a seed imbibition protein (SIP) with *O* -glycosyl hydrolase activity and plays a role in the biosynthetic and hydrolytic pathways of raffinose (Raf) metabolism, under abiotic stress condition. Raffinose scavenges hydroxyl radicals that leads to protect plant cells from oxidative damage caused under biotic and abiotic stress (Peters et al., 2010; Nishizawa et al., 2008).

The Methylenetetrahydrofolate reductase family protein proline dehydrogenase 1, PRODH (AT3G30775), was downregulated 12.73-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It an enzyme identified in the genome of *Arabidopsis*. Proline dehydrogenase (ProDH) catalyzes proline to delta-1-pyrroline-5-carboxylate (P5C). Proline is a proteinogenic amino acid that is involved in primary metabolism, redox homeostasis, protection against stress, and signaling in many prokaryotes, mammals and plants. Proline dehydrogenase (ProDH) can transfer electrons to the mitochondrial electron transport chain which regulates cellular redox states. It regulates the generation of ROS by the plasma membrane respaorty burst NADPH oxidase homolog D that influences early and late PTI responses (Cabassa-Hourton., et al., 2016; Fabro et al., 2016).

There are also some other transcripts in this experiment that were downregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants that play a role in the signal transduction networks to control the activation of defense responses in plants. Amongst them is the tetratricopetide-repeat thioredoxin-like 3 (TTL3) (AT2G42580) and tetratricopetide-repeat thioredoxin-like 4 (TTL4) (At3g58620) which were downregulated 6.02 and 3.31-fold, respectively in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They are member of the TTL family of proteins. The TTLs are plant-specific proteins that contain six TPR domains in specific positions throughout the sequence and a C-terminal sequence having homology to thioredoxins called the thioredoxin-like (TRXL) motif. The main characteristics of TRXL motif are that they do not contain Cys residues that are conserved in the other thioredoxins (Rosado., 2006; Ceserani., 2009). TTLs contain basic residues known as the carboxylate clamp in the TPR-binding domain. The carboxylate clamp domain interacts with the acidic side chains at the C-terminal ends of Hsp90 and Hsp70. TTL proteins interact as potential interactors of the Hsp90 and Hsp70 chaperone complexes (Carrigan., 2006; Prasad et al., 2010). It has been shown the HSP70/HSP90 play a role in plants to integrate their signals from their biotic and abiotic environments via stomatal regulation (Clément et al., 2010).

ATCMPG1 (AT1G66160) was downregulated 10.24-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of protein family recognized by four conserved amino acid residues [Cys, Met, Pro, and Gly (CMPG)] domain designating their family. It has been suggested that the *AtCMPG1* gene promoter containing a W-box, is a responsive element involved in the regulation pathogen defense-related genes as a transcriptional regulator or protein degradation factors (Heise et al., 2011).

The *Arabidopsis* subgroup III SnRK2 (AT4G33950) gene was downregulated 4.10-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a protein kinase (Sucrose nonfermenting1-related kinase2), also known as Open Stomata 1 [OST1). This protein is a serine/threonine protein kinase that acts as a central and positive regulator of the ABA signaling pathway (Nakashima., 2009; Fujii et al., 2011). SnRK2 interacts with and phosphorylates type-A response regulator 5 (ARR5), a negative regulator of cytokinin signaling, leading to promote ARR5 protein stability. Expressing of ARR5 enhanced ABA hypersensitivity and drought tolerance in *Arabidopsis* (Huang et al., 2018).

Azelaic acid induced 1 (AT4G12470) was downregulated 4.41-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. AZI1 was classified as lipid transfer from subfamily of EARLI1 gene. It contains a hydrophilic proline-rich domain (PRD) and a hydrophobic eight cysteine motif (8CM). It has been shown that AZI1 and DIR1 (another lipid transfer protein) are responsible for the transfer of signals related to SAR induced by azelaic acid (AZA) and glycerol-3-phosphate (Wang et al., 2016; Yu et al., 2013).

SPFH/Band 7/PHB domain-containing membrane-associated protein family, ATHIR2 (At3g01290), was downregulated 13.6-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of (HIR) gene family. They encode proteins of about 30 kD that contain the stomatin/prohibitin/flotillin/HflK/C (SPFH) domain, also called as the prohibitin (PHB) domain or band 7 domain. AtHIR2 is a part of the RPS2 immunity complex. Arabidopsis RPS2 is a NB-LRR resistance (R) protein, which indirectly recognizes the bacterial effector protein AvrRpt2 that activates effector triggered immunity (ETI). It has been suggested that the AtHIR proteins are localized in membrane microdomains and associated with RPS2 to mediate ETI response (Qi et al., 2011).

MDIS1-INTERACTING RECEPTOR LIKE KINASE2, MIK2 (AT4G08850), was downregulated 3.98-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a receptor that is involved in responses to cell wall perturbation. MIK2 plays a role with THE1, a putative cell wall integrity sensor (CWI) in response to cellulose biosynthesis inhibition. Moreover, it is essential for the control of normal root growth direction and salt tolerance in a THE1-dependent manner. Moreover, MIK2 has function in resistance to the fungal root pathogen *Fusarium oxysporum.* Therefore, it has been suggested that MIK2 plays a role in CWI sensing and affects various aspects of plant growth and development, as well as abiotic and biotic stress responses (Van der at al., 2017).

Sugar transporter 1, STP1 (AT1G11260), was downregulated 4.89-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is protein conserved among plants and is involved in hexose transport in cells of different tissues (203). Phosphorylation-sugar transporter 13 (STP13) affects antibacterial defense in *Arabidopsis thaliana*. STP13 associates with the flagellin receptor flagellin-sensitive 2 (FLS2) and its coreceptor BAK1. BAK1 phosphorylates STP13 at threonine 485, which promotes its monosaccharide uptake activity to compete with bacteria for extracellular sugars. Therefore, it decreases the availability of extracellular sugar for bacteria as an energy source. Regulation of sugar uptake can suppress bacterial proliferation and be a defense strategy against bacterial infection. It has been shown that STP1 and STP13 restrict bacterial proliferation in the apoplast by reducing sugar content during host-pathogen interactions (204).

The senescence-associated gene 21, SAG21 (AT4G02380), was downregulated 6.06-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It belongs the LEA

family protein *Arabidopsis* genome. It has been suggested (ATLEA5) involved in tolerance under abiotic stress (Yamada et al., 2016).

The BLUE COPPER BINDING PROTEIN, ATBCB (AT5G20230), was downregulated 9.8-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It involves in lignin biosynthesis. It was upregulated in response to the *Pseudomonas syringae* in *Arabidopsis* (Mishina et al., 2007).

The EARLY RESPONSE TO DEHYDRATION, 6ERD6 gene (AT1G08930), was downregulated 5.95-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It encodes a sugar transporter that is a member of a multigene family in the *Arabidopsis* genome. ERD6 gene was upregulated by dehydration in cold treatment (Kiyosue et al., 1998).

The TN13 is a TIR-NBS protein (AT3G04210) was downregulated 8.96-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is involved in basal resistance to *Pseudomonas syringae pv*. tomato (*Pst*) DC3000 lacking virulence effectors. It is located in the ER membrane and contains an N-terminal TM domain. TN13 associates with MOS6 and makes a complex at the cytoplasmic side of the ER through its C-terminal bipartite NLS. MOS6 acts as a shuttle for cargo proteins into the nucleus in response to the *Pseudomonas syringae pv.* tomato (Pst) DC3000 that lacks the effectors AvrPto and AvrPtoB in *Arabidopsis*. TN13 is released from the ER membrane by hypothetical protease after pathogen stimulus and imported to the nucleus, which leads to activation of defense response (Lüdke, et al., 2018).

Disease resistance protein (TIR-NBS-LRR class) (AT1G31540) and Disease resistance protein (TIR class) (AT1G61100) were downregulated 4.13-fold and 5.44-fold, respectively, in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They are the R protein that are involve in plant defense responses. They involve in the recognition of specialized

pathogen effectors called avirulence (Avr) proteins that trigger resistance pathways in plant (DeYoung et al., 2006).

The ARABIDOPSIS THALIANA SENESCENCE 1, ATSEN1 gene (AT4G35770), was downregulated 3.19-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It has been showen that SEN1 promoter binds the uidA reporter gene that has been confirmed to play a role in the SEN1 defense-signaling pathway. It also has been demonstrated that WRKY6 TF is a negative regulator of SEN1 (Schenk, et al., 2005; Robatzek., 2002).

The MAPK/ERK KINASE KINASE 3, MEKK3 gene (AT4G08470) was downregulated 3.2-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of MAPKs family proteins that plays an essential role in response to stimuli with several cellular and adaptive responses. The function of MEKK3 is still unknown. However, it is upregulated under abiotic stress condition (Danquah., 2014)

The leucine-rich repeat receptor kinase, MIK2 (AT4G088), was downregulated 3.97-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a regulator of cell wall damage responses triggered upon cellulose biosynthesis inhibition. MIK2 is essential for control of normal root growth and salt tolerance in a THE1-dependent manner. MIK2 plays a role in resistance to the fungal root pathogen *Fusarium oxysporum*. It has been suggested that involvement of MIK2 in CWI sensing has an impact on the regulation of various aspects of growth, as well as responses to abiotic and biotic stresses (Van der et al., 2017).

The calmodulin-binding protein (AT4G31000) was downregulated 5.83-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of a family of calmodulin-binding protein in *Arabidopsis*. Calcium is messenger in plants involved in mediating various signals including plant hormones, light, biotic and abiotic stresses. Complex

Ca/CaM regulates signal network that impacts plant development growth and responses to environmental stimuli. Cytosolic free Ca2+ concentrations increase in response to environmental stimuli that leads to the formation of active Ca2+/CaM complexes. This complex modulates cellular functions by interacting with regulatory proteins including transcription factors, protein kinases, and phosphatases, and with ion transporters that are involved in defense responses (Yang et al., 2003).

The leucine-rich repeat protein kinase family protein SUPPRESSOR OF BIR1 1, SOBIR1 (AT2G31880), was downregulated 5.71-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. An RLK acts as an adapter kinase for a number of tomatoes RLPs involved in plant immunity and is essential for the functions of Arabidopsis RLP1, RLP30 and RLP42 in PTI pathways. It has been found that BAK1 and SOBIR1 associate with each other in the absence of BIR1. BIR1 is as a negative regulator of plant immunity. Association of BAK1 and SOBIR1 promotes cell death and defense response (Liu et al., 2016).

The Phospholipase C1, ATPLC1 gene (AT5G58670), was downregulated 6.25-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It has a key role in various signal-transduction pathways. AtPLC1 represents a PI-PLC in *Arabidopsis*. AtPLC1 has a nonconserved short N-terminal region, whereas PI-PLC has a long N-terminal region. AtPLC1 contains the E-F hand and the X and Y domains. It has been suggested that AtPLCl may have a role in the signal-transduction pathways under environmental stresses condition (Hirayama et al., 1995).

The EXO70B2 gene (AT1G07000) was downregulated 7.42-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a subunit of exocyst complex. It has

been suggested that Exo70B2 and Exo70H1 play a role in response to pathogens. Exo70B2 interacts with sec5 that impacts on paplia formation (Pečenková et al., 2011).

The ubiquitin Ligase XBAT32 protein (AT4G14365) was downregulated 9.81-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is induced by auxin and play a role in lateral root modification (Nodzon., 2004).

The genes that have been mentioned were down regulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. Therefore, it can be suggested that down regulation of these genes is due to the impact of the ZnONP treatment on *Arabidopsis.*

3.2. Transcripts that were upregulated in response to the treatments

Of the 249 transcripts that were upregulated in response to the treatments, 2 transcripts were upregulated in ZnONP treated plants compared to untreated control plants and Zn^{+2} ion control plants. Seventy-eight transcripts were up regulated in ZnONP treated plants compared to Zn^{+2} ion control plants. Moreover, 189 transcripts were upregulated in untreated control plants compared to Zn^{+2} ion control plants.

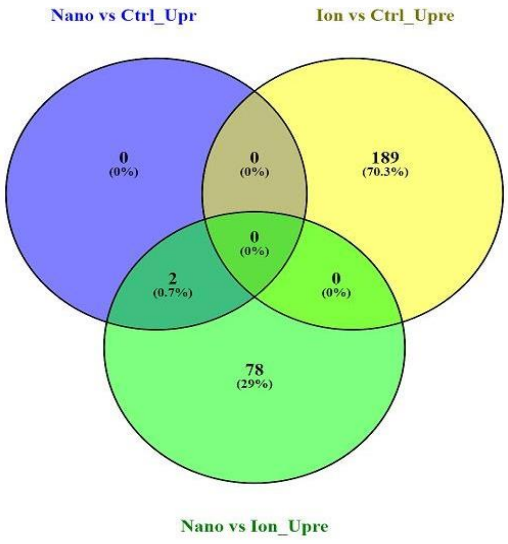

Fig. 7. Numbers of upregulated genes in response to the ZnONP treated plants, Zn^{+2} ion control plants and untreated control plants

3.2.1. Transcripts upregulated in ZnONP treated plants compared to untreated control plants and Zn $^{+2}$ ion treated control plants. (Appendix 4)

The OXIDATIVE STRESS 3, OXS3 gene (AT5G56550), was upregulated 3.14-fold the in ZnONP treated plants compared to the untreated control plants and 5.10-fold compared to the Zn $^{+2}$ ion treated control plants. It is a member of a family of proteins that share a putative N-acetyltransferase domain. It has been suggested it might protect plant from oxidative damage under stress condition (225). The ATNAP gene (AT1G69490) was upregulated 4.42-fold in ZnONP treated plants compared to the untreated control plants and 4.95-fold compared to the Zn $^{+2}$ ion treated control plants. It is a transcription factor of the NAC family genes that has role in leaf senescence. AtNAP repressed the expression of AREB1 under normal and salt stress conditions. ABSCISIC ACID–RESPONSIVE ELEMENT BINDING PROTEIN1 (AREB1) is a transcription factor that binds to the abscisic acid (ABA) responsive genes and promotes drought tolerance. It has been shown that AtNAP acts as a negative regulator of AREB1 in salt stress responses. Therefore, it has reduced the tolerance under abiotic stress (Seok et al., 2017).

3.2.2. Transcripts upregulated in response to the ZnONP treated plants compared to the Zn $^{+2}$ ion treated control plants. (Appendix 5 and 9)

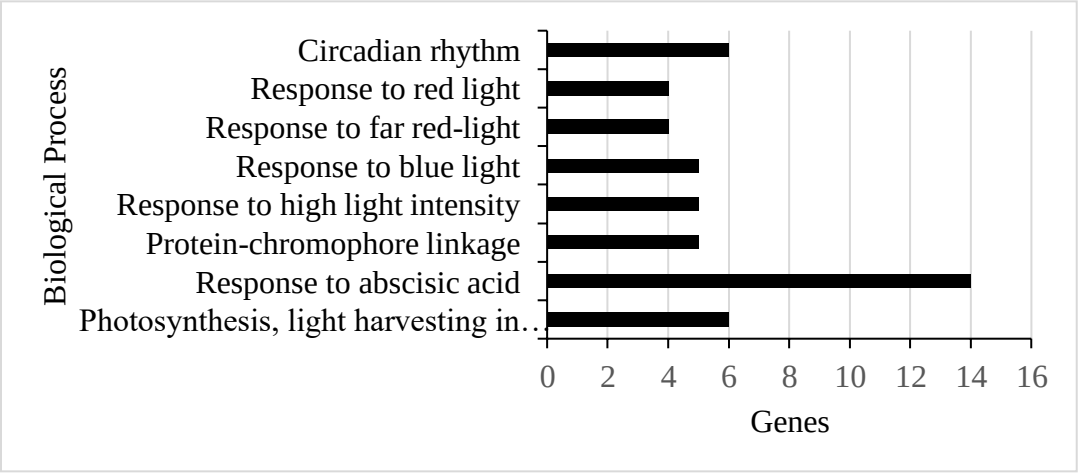

Fig. 8. Numbers of upregulated genes in response to ZnONP treated plants compared to the Zn $^{+2}$ ion control plants

The phytohormone ABA is as an endogenous messenger induced under biotic and abiotic stress responses. The ABA receptor are ABI1 (PP2C) and an ABA-binding RCAR member that form a heteromeric complex. The receptor complex controls ABA signaling located in the cytosol and the nucleus. The phosphatase activity of the PP2C prevents the action of the protein kinases OST1 and related SnRKs. Perception of ABA blocks the phosphatase activity of these receptors. Then the protein kinases are released and phosphorylate the key targets of the ABA signaling pathway. In the nucleus, key targets are the basic leucine zipper transcription factor ABI5 and ABFs (ABA responsive element (ABRE) binding factors). Phosphorylated ABFs binds to the ABA-responsive cis-element and regulates the ABA-responsive transcription. ABI3 binds to ABI5 to promote its action and regulates ABA-dependent gene expression (Raghavendra et al., 2010). In this experiment the genes that are involved in ABA pathway were upregulated in response to the ZnONP treated plants compared to the Zn^{+2} ion treated control plants based on the gene ontology analysis**.** Amongst them is the SENESCENCE ASSOCIATED GENE 113, SAG113 (At5g59220), that was upregulated 3.79-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It encodes a nuclear protein belonging to the protein phosphatases type 2C (PP2Cs) induced by ABA in *Arabidopsis* under stress condition (Zhang, et al., 2013). HAI-1 is a negative regulator of ABA signal transduction. It has been observed that up-regulation of SAG113 under high ABA levels promoted stomatal opening, rather than closure, in *Arabidopsis*. Therefore, it has been suggested SAG113 is involved in stress recovery signaling network under biotic stress (Yeung et al., 2018).

The ARABIDOPSIS THALIANA HOMEOBOX 7, ATHB-7 gene (AT2G46680), was upregulated 4.25-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a transcription factor (TFs) that belongs to the homeodomain-leucine zipper

subfamily I (HD-Zip I) (Ré et al. 2014). ATHB7 is a positive transcriptional regulator of PP2C genes, and act as negative regulators of abscisic acid signaling. ATHB7 is a transcript repressor of genes encoding the ABA receptors PYL5and PYL8 in response to an ABA stimulus. It has a negative feedback effect on ABA signaling in response to abiotic stress (Valdés et al., 2012).

The ABI five binding protein 3 (AT3G29575) was upregulated 3.05-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is involved in ABA signaling and stress response in *Arabidopsis*. It has been shown in ABI5 was induced at high level of ABA under stress condition that prevent growth inhibition in *Arabidopsis* (Garcia et al., 2008).

The Galactinol synthase 2, ATGOLS2 gene (AT1G56600), was upregulated 8.40-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is an enzyme that is involved in the synthesis of the raffinose family oligosaccharides. It has been found that galactinol and raffinose scavenge hydroxyl radicals that lead to protect plant cells from oxidative damage under stress condition (Nishizawa., 2008).

The Multiprotein bridging factor 1C, ATMBF1C gene (AT3G24500), was upregulated 3.77-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a transcriptional factor that increases the transcription of its target genes by making the complex between transcription factors (TFs) and TATA-box binding protein (TBP) (234). It has been shown that overexpression of ATMBF1C promoted the tolerance against abiotic and biotic stresses in *Arabidopsis* (Kim et al., 2007).

The low-temperature-induced 78, RD29A gene (AT5G52310) was upregulated 3.31-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a hydrophilic protein that contains two cis acting elements ABRE (ABA-responsive element) and DREBs (DREbinding proteins). The expression of RD29A is via the ABA-dependent and ABA-

independent pathways. It has been shown that it was upregulated in response to ABA treatment and abiotic stress condition to promote the tolerance of the plant under changing environmental condition (Yamaguchi-Shinozakiaib et al., 2010; Yang, et al., 2011)

The PSI1 and PSII proteins with different absorption spectra are part of light harvesting complexes that convert light energy into chemical energy in the photosynthesis process. State transitions happen in three steps. Upon excitation of PSII, electron flow between PSI and PSII lead to over-reduction of the plastoquinone (PQ) pool and initiate kinases activation. Activated kinases phosphorylate the light harvesting complex II (LHCII). There are three major LHCII proteins including Lhcb1, Lhcb2, and Lhcb3. The phosphorylation of LHCII leads to dissociate from PSI, which is the third step of state transitions (Kouřil et al., 2005). In this experiment, the gene that is involved in the light harvesting complexes were upregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They include Chlorophyll B-binding protein 3, the LHCB2 gene (AT5G54270) and photosystem II light harvesting complex protein 2.2 (AT2G05100) and LHCB1 genes (At1g29920) which were upregulated 3.37, 3.67 and 3.54-fold, respectively, in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. They contain N-terminal transit peptide that are synthesized in the cytoplasm as soluble precursors and imported into the chloroplasts (Kouřil et al., 2005; Jackowski et al., 2001). Light-harvesting complex II (LHCII) is an important component of the photosynthetic system that has role in light capture and acclimation to changing light. Lhcb1, Lhcb2, and Lhcb3 are trimer isoforms of LHCII. Association of an LHCII trimer with PSI in the PSI-LHCII supercomplex is dependent of the LHCII phosphorylation mediated by the kinase STATE TRANSITION. Only Lhcb1 and Lhcb2 can be phosphorylated in the N-terminal region. It has been shown that the differential phosphorylation leads them to play contrasting roles in light acclimation during

photosynthesis (Longoni et al., 2015). It has been showed that over-expression of a LHCB member enhances stomatal sensitivity to ABA. Therefore, they might be involved in ABA signaling in response to stress condition in *Arabidopsis* (Xu et al., 2011).

Plants receives light from the environment that enable them to manage their growth and development. They perceive light through various types of photoreceptors such as phytochromes, which are in an inactive or active form. The balance between the two forms is dependent on R/FR proportions in deferent developmental process. Proportion of R/FR affects different developmental processes such as seed germination, stem elongation, leaf development, bud outgrowth, flowering, photosynthesis and tolerance of the plants under biotic and abiotic stress condition (Demotes-Mainard et al., 2016). Low R/FR reduced the transcription of the genes that involved in the SA pathway (de De et al., 2013). In this experiment, the gene involved in response to far red light were upregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants based on the gene ontology analysis. Amongst them is the pseudo-response regulator 7, the PRR7 gene (AT5G02810), that was up-regulated 3.82-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a member of a family of PRR genes that has a role in the *Arabidopsis* circadian clock. They contain a receiver-like (or pseudo-receiver) domain at their N-terminus and a 50 amino acids C-terminal domain called the CCT motif (Mizuno et al., 2005). PRR7 regulates the expression of genes involved in abiotic stress stimuli. It has been suggested that PRR7 involved in low temperature and drought responses. Liu et al 2013 showed that PRR7 mutants affect the ABA promotion of stomata closing (Liu et al., 2013). It has also been observed that *prrs* triple mutants showed more tolerance to low temperature and drought stress conditions as compared to wild-type plants (Nakamichi et al., 2009).

Blue light which mediates phototropic response in plants fall into two receptor families which include the cryptochromes and phototropins. They play a role in photomorphogenic responses, such as regulation of cell elongation and photoperiodic flowering. The absorption of the red/far-red light receptors (phytochromes) and the blue light receptors (cryptochromes and phototropins) regulate the control of development and energy production in plants (Lin., 2002). In this experiment, the genes that response to blue light spectrum were upregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants based on the gene ontology analysis. Amongst them is the GIGANTEA gene (AT1G22770) that was upregulated 11.47-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It functions in leaf movements, hypocotyl elongation, and photoperiodic control of flowering in *Arabidopsis* (Sothern et al., 2002). It has been shown that overexpression of GI leads to accelerate flowering time and reduce the plants tolerance under salt stress conditions (Kim et al., 2013).

The Flavin-binding kelch repeat f box 1 (AT1G68050) was upregulated 3.04-fold in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. It is a blue-light receptor and a key component of the SKP1/CUL1/F-box (SCF)-type E3 ligase complex. It contains three domains including LOV, F-box, and KELCH-repeat. FKF1 interacts with proteins GIGANTEA (GI) and ZEITLUPE (ZTL) that are involved in CO stability regulation. The CONSTANS (CO) protein plays a role in expression of FLOWERING LOCUS T (FT) during long days (Song et al., 2014).

The genes that has been mentioned above were upregulated in the ZnONP treated plants compared to the Zn^{+2} ion treated control plants. Therefore, it can be concluded that the deferential expression of these genes was due to the ZnONP treatment of *Arabidopsis.*

4. CONCLUSION AND DISCUSSION

The evaluation of the impact that ZnONPs (10 ug/L) had on *Arabidopsis* gene expression compared to ionic Zn and untreated control plants revealed that more genes were downregulated in specific response to the ZnONPs than upregulated with the majority of the genes being involved in biotic and abiotic stress responses. These data suggest that ZnONP-Arabidopsis interactions result in greater suppression of defense and stress response related genes. Thus, the extended exposure of *Arabidopsis* plants to ZnONPs had a larger effect on the suppression of genes involved in stress and environmental stimuli response suggesting that extended plant exposure of ZnONP may adversely affect the plants ability to respond to biotic and abiotic stresses.

The ZnONPs treatment compared to ionic Zn and untreated plants induced the down regulation of genes involved in the defense response indicated that prioritization of plant response to stress condition under exposer to ZnoNP. These genes include Cytochrome P450 (CYP81D8) (AT4G37370), Heavy metal transport/detoxification superfamily protein (AT5G52750), Phospholipase A 2A (AT2G26560) , Legume lectin family protein (AT3G16530), azelaic acid induced 1, AZI (AT4G12470), GLYCINE RICH PROTEIN 9 (AT2G05440), and the expressed protein (AT3G01345). Moreover, ZnONPs treatment compared to untreated plants induced the down regulation of genes involved in defense response including AZI3 (AT4G12490), Jacalin-related lectin 23 (JAL23) (AT2G39330), S locus-related glycoprotein 1 (SLR1) (AT3G05727), Thionin 2.1 ,PR-13 family of pathogen related protein (AT1G72260) and also the genes involved in oxidative stress including Glutathione S-transferase 6 (AT1G02930), Pollen Ole e 1 allergen and extensin family protein (AT3G16670) and finally the genes involved in hormone signaling path ways such as sulfotransferase 12

(AT2G03760) involved in brassinosteroid metabolic process and ATP binding cassette subfamily B4 (AT2G47000) involved in auxin efflux mechanism.

In addition, ZnONPs treatment compared to ionic Zn induced the down regulation of genes involved in the response to xylogucan metabolite process such as to Ionic Zn^{2+} were xyloglucan endotransglucosylase/hydrolase 18 (AT4G30280), xyloglucan endotransglucosylase/hydrolase 4(AT2G06850), xyloglucan endotransglucosylase/hydrolase 24(AT4G30270), Xyloglucan endotransglucosylase/hydrolase family protein (AT5G57560), xyloglucan:xyloglucosyl transferase 33 (AT1G10550) and xyloglucan endotransglucosylase/hydrolase 16 (AT3G23730), xyloglucosyl transferase 33 (AT1G10550) and also the genes that has function in cell wall biogenesis and cell wall organization including (LRR) family protein (LRX3) (AT4G13340) that has role in plant development and particularly cell wall formation, Plant invertase/pectin methylesterase inhibitor (ATPME41), Expansin-like A2 (AT4G38400) (AtEXLA2).

There was also the downregulation of hormone-responsive genes observed such as LURP-one-like protein (LURP1) (DUF567) AT2G14560, Cysteine-rich RLK (RECEPTOR-like protein kinase) CRK19 (AT4G23270) Ankyrin repeat family protein (ACCELERATED CELL DEATH 6) (ACD6) (AT4G14400), Ankyrin (BDA1) (AT5G54610), WRKY DNA-binding protein 18 (AT4G31800), *WRKY25* (At2g30250) that are involved in SA signaling pathway. Moreover, there were down regulated transcripts that play a role in BRASSINOSTEROID-SIGNALLING pathway such as Somatic embryogenesis receptor-like kinase 4 (AT2G13790) (SERK4), BZS1, BZS1 (BBX20) (At4g39070), EXO (EXORDIUM) (AT4G08950). Finally, The down regulated genes that have roles in defense response such as wall-associated kinase 2(AT1G21270) WAK2 with a function in cell expansion under environmental stimuli such as

pathogen and wounding, AtIQM1 (AT4G33050) IQM1 with function in promoting root growth, and stomatal opening or prevent stomatal closure, DREB26 with role in plant growth and development and in defense response, FLAGELLIN-SENSITIVE 2(AT5G46330) (FLS2) that has role in immune response against pathogen, Proline dehydrogenase (ProDH) participate in electron transfer system in response to generation of ROS, HIR2 that mediate ETI response, MIK2(AT4G08850) that impact on plant growth development, as well as abiotic and biotic stresses responses, sugar transporter 1 (AT1G11260) (STP1) involved in defense strategy against bacterial infection, senescence-associated gene 21(AT4G02380) that has function in in tolerance under abiotic stress, TN13 is a TIR-NBS protein (AT3G04210) has role in activation of basal defense response, *ATSIP2* (At3g57520) protect plants against oxidative damage caused under biotic and abiotic stress condition, MYB protein is a transcription factor that participated in regulates PR gene expression, GATA-type zinc finger transcription factor involvement in in plant defense response, Leucine-rich repeat protein kinase family protein (AT2G31880) is a RLK involvement that promotes cell death and defense response under biotic stress response.

Differentially up-regulated genes in nZnO treatment compared to ionic Zn and untreated plants were ATNAP (ANAC029) (AT1G69490) that has role in leaf sencene. And OXIDATIVE STRESS 3(OXS3) (AT5G56550) that protect plants from oxidative damage. Moreover, up-regulated genes in nZnO treatment compared to ionic Zn based on the gene ontology (GO) were involved in abscisic acid signaling pathway SENESCENCE ASSOCIATED GENE 113 (SAG113) (HAI1) (At5g59220) has role in stress recovery biotic stress, ABI five binding protein 3 (AT3G29575) that prevent growth inhibition in Arabidopsis under stress condition, Stress-responsive protein (KIN1) that induce under drought and law temperature condition, Multiprotein bridging factor 1C (ATMBF1C) (AT3G24500) that enhances the tolerance against

abiotic and biotic in *Arabidopsis,* RD29A (AT5G52310) that promotes the tolerance of the plant under changing environmental condition.

The other genes that were upregulated in ZnONPs treatment compared to ionic Zn play a role in light harvesting complex, photosynthesis, response to blue light, response to far red light and response to high light intensity. They include Chlorophyll B-binding protein 3 (LHCB3) (AT5G54270) and (AT2G05070) (LHCB2) and LHCB1 (At1g29920) that involve in ABA signaling in response to stress condition in Arabidopsis, GIGANTEA (AT1G22770) that accelerate flowering time and hypocotyl elongation, Flavin-binding kelch repeat f box 1 (AT1G68050) that play a role FLOWERING LOCUS T (FT) in long days.

Landa et al., 2012 showed exposure 100mg/l nZno to *Arabidopsis* after 7 days induced the up regulation of genes that involved in salt, oxidative, water and osmotic stress. In addition, the up regulation of genes involved in wounding and defend against pathogen were observed that indicate in their experiment nZno damaged the plant tissue. Moreover, lateral root development was observed indicating that plants initiated new root growth under stress condition. In addition, the activation of genes involved in oxidative stress response nZno treatment enhanced the generation of ROS in exposed roots. They also showed that the genes that were involved in light harvesting complex, election transport system in photosystem I and II, cell wall biogenesis, cell wall organization were downregulated. They also showed that exposure nTio2 to Arabidopsis plants increase the activation of genes involved in oxidative stress (Landa *et al.,* 2015). These finding are inconsistent with our observation that showed the down regulation of defence response genes and up-regulation of light harvesting complex genes and photosynthesis responses genes. However. the downregulation of the genes involved in cell wall organization

and cell wall biogenesis agree with our observation that showed this result in nZnO compared to ionic Zn^{+2}.

Ze et al., 2011 showed that nTio2 promoted the expression of light-harvesting complex II (LHCII) b and LHCII II on the thylakoid membrane in *A. thaliana*. Moreover, the absorption peak intensity of the chloroplast in red and blue region were increased. This finding agrees with our result that showed the exposure of nZnO in comparison with ionic Zn^{+2} could increase the light absorption of chloroplast, regulate the distribution of light energy from PS I to PS II by enhancing LHCII and accelerate the transformation from light energy to electronic energy, water photolysis, and oxygen evolution (Ze *et a*l., 2011).

Kaveh et al., 2013 also analyzed the expression of genes in *Arabidopsis thaliana* in response to AgNPs and silver ions (Ag+). They observed that the up-regulated genes were involved in the response to metals and oxidative stress, vacuolar cation/proton exchanger, superoxide dismutase, cytochrome P450-dependent oxidase, and the down-regulated genes were involved in response to pathogens and hormonal stimuli such as auxin regulated gene ethylene signaling pathway, and systemic acquired resistance (SAR) against fungi and bacteria. The downregulation of hormone-responsive genes indicates efforts by the plant to limit the growth under stress conditions. They also have function under biotic and abiotic stress condition (Kaveh *et al.* 2013). These result are consistent with our observation that showed the down regulation of pathogens responsive genes and hormonal stimuli and inconsistent with our result that showed the down regulation of metals and oxidative stress responsive genes such as cytochrome P450-dependent oxidase in exposure of nZnO in comparison with ionic Zn^{+2} in *Arabidopsis thaliana*.

Khodakovskaya et al., 2010 showed that multiwall carbon nanotubes in tomato induce the expression of cellular responses, stress responses, transport, signal transduction, and metabolic

and biosynthetic process genes (254). However, in this experiment our observation showed the downregulation of stress responsive genes in the exposer of zinc oxide nanoparticles.

Shen et al., 2010 also showed that exposure of Single-walled carbon nanotubes (SWCNTs) on Arabidopsis induce ROS accumulation, chromatin condensation and induction of programmed cell death, however induction of stress-related genes was increased at 24 h in SWCNT-injected plants. However, differential expression of these genes was not observed after 36 h and macroscopic cell death at the tissue level was not observed after the SWCNT treatments, showing that SWCNTs induced limited cell injury (Shen *et al*., 2010). These observations agree with our result that we did not have program cell death. However, we found the downregulation of stress responsive genes in exposure of nZno nanoparticles in *Arabidopsis* plants.

In summary in this experiment, most of the downregulated transcripts were involved in response to pathogens, hormonal stimuli such as Salicylic acid (SA), Brassinosteroid (BR), Cell wall biogenesis and cell wall organization. As hormones are involved primarily in the regulation of plant development, and downregulation of hormone-responsive genes may simply reflect attempts of the plant to limit the growth stress condition. Moreover, downregulated of genes involved in cell organization and biogenesis, suggests alterations in cell division and cell structure. In addition, the up-regulated transcripts involved mainly stress responsive genes participating in defense against abiotic (oxidative stress, cold, salt, water deprivation), hormonal stimuli such as abscisic acid and light harvesting complexes and electron transport systems in photosystem I and II that reflect the attempt of the plants in stress recovery and also manage their growth and development under changing environmental condition.

The reason that our results were inconsistent with some other result is the plant uptake of nanoparticles is affected by several factors including the concentration, type of nanoparticles, the size of nanoparticle and also we grew our plants in tissue culture and then we analyzed the differential expression of genes after harvesting the plants at the rosette stage.

LITERATURE CITED

Adhikari, Tapan, S. Kundu, and A. Subba Rao. "Zinc delivery to plants through seed coating with nano-zinc oxide particles." *Journal of plant nutrition* 39, no. 1 (2016): 136-146.

Agrios GN (1997) Plant Pathology, Ed 4. Academic Press, San Diego, pp 46–52.

Albrecht, Catherine, Eugenia Russinova, Birgit Kemmerling, Mark Kwaaitaal, and Sacco C. de Vries. "Arabidopsis somatic embryogenesis receptor kinase proteins serve brassinosteroid-dependent and-independent signaling pathways." *Plant physiology* 148, no. 1 (2008): 611-619.

Almeelbi, Talal, and Achintya Bezbaruah. "Nanoparticle-sorbed phosphate: iron and phosphate bioavailability studies with Spinacia oleracea and Selenastrum capricornutum." *ACS Sustainable Chemistry & Engineering* 2, no. 7 (2014): 1625-1632.

Alidoust, Darioush, and Akihiro Isoda. "Phytotoxicity assessment of γ-Fe 2 O 3 nanoparticles on root elongation and growth of rice plant." *Environmental earth sciences* 71, no. 12 (2014): 5173-5182.

Alidoust, Darioush, and Akihiro Isoda. "Effect of γFe 2 O 3 nanoparticles on photosynthetic characteristic of soybean (Glycine max (L.) Merr.): foliar spray versus soil amendment." *Acta physiologiae plantarum* 35, no. 12 (2013): 3365-3375.

Armijo, Grace, Paula Salinas, Mariela Inés Monteoliva, Aldo Seguel, Consuelo García, Eva Villarroel-Candia, Wei Song, Alexander Ronald van der Krol, María Elena Álvarez, and Loreto Holuigue. "A salicylic acid–induced lectin-like protein plays a positive role in the effector-triggered immunity response of Arabidopsis thaliana to Pseudomonas syringae Avr-Rpm1." *Molecular plant-microbe interactions* 26, no. 12 (2013): 1395-1406.

Arason, Guđmundur Jóhann. "Lectins as defence molecules in vertebrates and invertebrates." *Fish & Shellfish Immunology* 6, no. 4 (1996): 277-289.

Arora, Sandeep, Priyadarshini Sharma, Sumit Kumar, Rajeev Nayan, P. K. Khanna, and M. G. H. Zaidi. "Gold-nanoparticle induced enhancement in growth and seed yield of Brassica juncea." *Plant growth regulation* 66, no. 3 (2012): 303-310.

Asai, Tsuneaki, Guillaume Tena, Joulia Plotnikova, Matthew R. Willmann, Wan-Ling Chiu, Lourdes Gomez-Gomez, Thomas Boller, Frederick M. Ausubel, and Jen Sheen. "MAP kinase signalling cascade in Arabidopsis innate immunity." *Nature*415, no. 6875 (2002): 977.

Auffan, Mélanie, Jérôme Rose, Mark R. Wiesner, and Jean-Yves Bottero. "Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro." *Environmental Pollution* 157, no. 4 (2009): 1127-1133

Auffan, Mélanie, Jérôme Rose, Mark R. Wiesner, and Jean-Yves Bottero. "Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro." *Environmental Pollution* 157, no. 4 (2009): 1127-1133.

Baumberger, N., M. Steiner, Ulrich Ryser, B. Keller, and C. Ringli. "Synergistic interaction of the two paralogous Arabidopsis genes LRX1 and LRX2 in cell wall formation during root hair development." *The Plant Journal* 35, no. 1 (2003): 71-81.

Bethke, Gerit, Rachael E. Grundman, Suma Sreekanta, William Truman, Fumiaki Katagiri, and Jane Glazebrook. "Arabidopsis Pectin Methylesterases contribute to immunity against Pseudomonas syringae." *Plant Physiology*164, no. 2 (2014): 1093-1107.

Bhatt, Indu, and Bhumi Nath Tripathi. "Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment." *Chemosphere* 82, no. 3 (2011): 308-317.

Blanvillain, Robert, Jong Heon Kim, Shimei Wu, Amparo Lima, and David W. Ow. "Oxidative stress 3 is a chromatin-associated factor involved in tolerance to heavy metals and oxidative stress." *The Plant Journal* 57, no. 4 (2009): 654-665.

Blanco, Francisca, Virginia Garretón, Nicolas Frey, Calixto Dominguez, Tomas Perez-Acle, Dominique Van der Straeten, Xavier Jordana, and Loreto Holuigue. "Identification of NPR1-dependent and independent genes early induced by salicylic acid treatment in Arabidopsis." *Plant molecular biology* 59, no. 6 (2005): 927-944.

Boller, Thomas, and Georg Felix. "A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors." *Annual review of plant biology* 60 (2009): 379-406.

Bosch, Maurice, Claus-Dieter Mayer, Alan Cookson, and Iain S. Donnison. "Identification of genes involved in cell wall biogenesis in grasses by differential gene expression profiling of elongating and non-elongating maize internodes." *Journal of experimental botany* 62, no. 10 (2011): 3545-3561.

Boller, T. (1995) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46,** 189–214

Boron, Agnieszka Karolina, Bram Van Loock, Dmitry Suslov, Marios Nektarios Markakis, Jean-Pierre Verbelen, and Kris Vissenberg. "Over-expression of AtEXLA2 alters etiolated arabidopsis hypocotyl growth." *Annals of botany* 115, no. 1 (2014): 67-80.

Botella. "The Arabidopsis tetratricopeptide thioredoxin-like gene family is required for osmotic stress tolerance and male sporogenesis." *Plant physiology* 158, no. 3 (2012): 1252-12 Camardella, Laura, Vito Carratore, Maria A. Ciardiello, Luigi Servillo, Ciro Balestrieri, and Alfonso Giovane. "Kiwi protein inhibitor of pectin methylesterase: Amino-acid sequence and structural importance of two disulfide bridges." *European journal of biochemistry* 267, no. 14 (2000): 4561-4565.

Cartagena, Joyce A., Sachihiro Matsunaga, Motoaki Seki, Daisuke Kurihara, Masami Yokoyama, Kazuo Shinozaki, Satoru Fujimoto, Yoshitaka Azumi, Susumu Uchiyama, and Kiichi Fukui. "The Arabidopsis SDG4 contributes to the regulation of pollen tube growth by methylation of histone H3 lysines 4 and 36 in mature pollen." *Developmental biology*315, no. 2 (2008): 355-368.

Cavalier, David M., Olivier Lerouxel, Lutz Neumetzler, Kazuchika Yamauchi, Antje Reinecke, Glenn Freshour, Olga A. Zabotina et al. "Disrupting two Arabidopsis thaliana xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component." *The Plant Cell* 20, no. 6 (2008): 1519-1537.

Carrigan, Patricia E., Laura A. Sikkink, David F. Smith, and Marina Ramirez‐Alvarado. "Domain: domain interactions within Hop, the Hsp70/Hsp90 organizing protein, are required for protein stability and structure." *Protein science* 15, no. 3 (2006): 522-532.

Cabassa-Hourton, Cécile, Peter Schertl, Marianne Bordenave-Jacquemin, Kaouthar Saadallah, Anne Guivarc'h, Sandrine Lebreton, Séverine Planchais et al. "Proteomic and functional analysis of proline dehydrogenase 1 link proline catabolism to mitochondrial electron transport in Arabidopsis thaliana." *Biochemical Journal* 473, no. 17 (2016): 2623-2634

Cecchini, Nicolás M., Kevin Steffes, Michael R. Schläppi, Andrew N. Gifford, and Jean T. Greenberg. "Arabidopsis AZI1 family proteins mediate signal mobilization for systemic defence priming." *Nature communications* 6 (2015): 7658.

Chapple, Clint. "Molecular-genetic analysis of plant cytochrome P450-dependent monooxygenases." *Annual review of plant biology* 49, no. 1 (1998): 311-343

Chen, Kegui, Baofang Fan, Liqun Du, and Zhixiang Chen. "Activation of hypersensitive cell death by pathogen-induced receptor-like protein kinases from Arabidopsis." *Plant molecular biology* 56, no. 2 (2004): 271-283.

Chen, An-Ping, Nai-Qin Zhong, Zhan-Liang Qu, Fang Wang, Ning Liu, and Gui-Xian Xia. "Root and vascular tissue-specific expression of glycine-rich protein AtGRP9 and its interaction with AtCAD5, a cinnamyl alcohol dehydrogenase, in Arabidopsis thaliana." *Journal of plant research* 120, no. 2 (2007): 337-343.

Chisholm, Stephen T., Gitta Coaker, Brad Day, and Brian J. Staskawicz. "Host-microbe interactions: shaping the evolution of the plant immune response." *Cell* 124, no. 4 (2006): 803-814.

Cho, Misuk, Zee-Won Lee, and Hyung-Taeg Cho. "ATP-binding cassette B4, an auxin-efflux transporter, stably associates with the plasma membrane and shows distinctive intracellular trafficking from that of PIN-FORMED proteins." *Plant physiology* 159, no. 2 (2012): 642-654.

Cocuron, Jean-Christophe, Olivier Lerouxel, Georgia Drakakaki, Ana P. Alonso, Aaron H. Liepman, Kenneth Keegstra, Natasha Raikhel, and Curtis G. Wilkerson. "A gene from the

cellulose synthase-like C family encodes a β-1, 4 glucan synthase." *Proceedings of the National Academy of Sciences* 104, no. 20 (2007): 8550-8555.

Coll, N. S., P. Epple, and J. L. Dangl. "Programmed cell death in the plant immune system." *Cell death and differentiation* 18, no. 8 (2011): 1247.

Cosgrove, Daniel J. "Loosening of plant cell walls by expansins." *Nature* 407, no. 6802 (2000): 321.

Cordoba, Elizabeth, Denise Lizeth Aceves-Zamudio, Alma Fabiola Hernández-Bernal, Maricela Ramos-Vega, and Patricia León. "Sugar regulation of SUGAR TRANSPORTER PROTEIN 1 (STP1) expression in Arabidopsis thaliana." *Journal of experimental botany* 66, no. 1 (2014): 147-159.

Clément, Mathilde, Nathalie Leonhardt, Marie-Jo Droillard, Ilja Reiter, Jean-Luc Montillet, Bernard Genty, Christiane Lauriere, Laurent Nussaume, and Laurent D. Noël. "The cytosolic/nuclear HSC70 and HSP90 molecular chaperones are important for stomatal closure and modulate abscisic acid-dependent physiological responses in Arabidopsis." *Plant physiology* 156, no. 3 (2011): 1481-1492.

Clouse, Steven D. "Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development." *The Plant Cell* 23, no. 4 (2011): 1219-1230.

Day, Brad, Douglas Dahlbeck, and Brian J. Staskawicz. "NDR1 interaction with RIN4 mediates the differential activation of multiple disease resistance pathways in Arabidopsis." *The Plant Cell* 18, no. 10 (2006): 2782-2791.

Davletova, Sholpan, Karen Schlauch, Jesse Coutu, and Ron Mittler. "The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis." *Plant physiology* 139, no. 2 (2005): 847-856.

Danquah, Agyemang, Axel de Zelicourt, Jean Colcombet, and Heribert Hirt. "The role of ABA and MAPK signaling pathways in plant abiotic stress responses." *Biotechnology advances* 32, no. 1 (2014): 40-52.

de la Rosa, Guadalupe, Martha Laura López-Moreno, David de Haro, Cristian E. Botez, José R. Peralta-Videa, and Jorge L. Gardea-Torresdey. "Effects of ZnO nanoparticles in alfalfa, tomato, and cucumber at the germination stage: root development and X-ray absorption spectroscopy studies." *Pure and Applied Chemistry* 85, no. 12 (2013): 2161-2174.

de De Wit, M., S. H. Spoel, G. F. Sanchez-Perez, C. M. M. Gommers, C. M. J. Pieterse, L. A. C. J. Voesenek, and R. Pierik. "Perception of low red: far-red ratio comprises both salicylic acid-and jasmonic acid-dependent pathogen defences in Arabidopsis." *Plant Journal* 75 (2013): 90.

DeYoung, Brody J., and Roger W. Innes. "Plant NBS-LRR proteins in pathogen sensing and host defense." *Nature immunology* 7, no. 12 (2006): 1243

Demotes-Mainard, Sabine, Thomas Péron, Adrien Corot, Jessica Bertheloot, José Le Gourrierec, Sandrine Pelleschi-Travier, Laurent Crespel et al. "Plant responses to red and far-red lights, applications in horticulture." *Environmental and Experimental Botany* 121 (2016): 4-21.

Denancé, Nicolas, Andrea Sánchez-Vallet, Deborah Goffner, and Antonio Molina. "Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs." *Frontiers in plant science* 4 (2013): 155.

Draeger, Christian, Tohnyui Ndinyanka Fabrice, Emilie Gineau, Grégory Mouille, Benjamin M. Kuhn, Isabel Moller, Marie-Therese Abdou et al. "Arabidopsis leucine-rich repeat extensin (LRX) proteins modify cell wall composition and influence plant growth." *BMC plant biology* 15, no. 1 (2015): 155.

El-Temsah, Yehia Sayed, and Erik J. Joner. "Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil." *Environmental toxicology* 27, no. 1 (2012): 42-49.

Epple, Petra, Klaus Apel, and Holger Bohlmann. "Overexpression of an endogenous thionin enhances resistance of Arabidopsis against Fusarium oxysporum." *The Plant Cell* 9, no. 4 (1997): 509-520.

Eulgem, Thomas, Victor J. Weigman, Hur-Song Chang, John M. McDowell, Eric B. Holub, Jane Glazebrook, Tong Zhu, and Jeffery L. Dangl. "Gene expression signatures from three genetically separable resistance gene signaling pathways for downy mildew resistance." *Plant Physiology* 135, no. 2 (2004): 1129-1144.

Fabro, Georgina, Yanina Soledad Rizzi, and María Elena Alvarez. "Arabidopsis proline dehydrogenase contributes to flagellin-mediated PAMP-triggered immunity by affecting RBOHD." *Molecular Plant-Microbe Interactions* 29, no. 8 (2016): 620-628.

Feng, Xiao-Ming, Yu Qiao, Ke Mao, and Yu-Jin Hao. "Ectopic overexpression of AtmiR398b gene in tobacco influences seed germination and seedling growth." *Plant Cell, Tissue and Organ Culture (PCTOC)* 102, no. 1 (2010): 53-59.

García-Gómez, C., M. Babin, A. Obrador, J. M. Álvarez, and M. D. Fernández. "Integrating ecotoxicity and chemical approaches to compare the effects of ZnO nanoparticles, ZnO bulk, and ZnCl 2 on plants and microorganisms in a natural soil." *Environmental Science and Pollution Research* 22, no. 21 (2015): 16803-16813.

Garcia, Mary Emily, Tim Lynch, Julian Peeters, Chris Snowden, and Ruth Finkelstein. "A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating Arabidopsis seeds and seedlings." *Plant molecular biology* 67, no. 6 (2008): 643-658.

Gou, Xiaoping, Hongju Yin, Kai He, Junbo Du, Jing Yi, Shengbao Xu, Honghui Lin, Steven D. Clouse, and Jia Li. "Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling." *PLoS genetics* 8, no. 1 (2012): e1002452.

Guo, Hongqing, Lei Li, Huaxun Ye, Xiaofei Yu, Alexandria Algreen, and Yanhai Yin. "Three related receptor-like kinases are required for optimal cell elongation in Arabidopsis thaliana." *Proceedings of the National Academy of Sciences*106, no. 18 (2009): 7648-7653

Gruyer, N., M. Dorais, C. Bastien, N. Dassylva, and G. Triffault-Bouchet. "Interaction between silver nanoparticles and plant growth." In *International Symposium on New Technologies for Environment Control, Energy-Saving and Crop Production in Greenhouse and Plant 1037*, pp. 795-800. 2013.

Gui, Xin, Yingqing Deng, Yukui Rui, Binbin Gao, Wenhe Luo, Shili Chen, Xuguang Li et al. "Response difference of transgenic and conventional rice (Oryza sativa) to nanoparticles (γFe 2 O " *Environmental Science and Pollution Research* 22, no. 22 (2015): 17716-17723.

Handy, Richard D., Frank Von der Kammer, Jamie R. Lead, Martin Hassellöv, Richard Owen, and Mark Crane. "The ecotoxicology and chemistry of manufactured nanoparticles." *Ecotoxicology* 17, no. 4 (2008): 287-314.

Hao, Dongyun, Masaru Ohme-Takagi, and Akinori Sarai. "Unique mode of GCC box recognition by the DNA-binding domain of ethylene-responsive element-binding factor (ERF domain) in plant." *Journal of Biological Chemistry* 273, no. 41 (1998): 26857-26861

Harholt, Jesper, Anongpat Suttangkakul, and Henrik Vibe Scheller. "Biosynthesis of pectin." *Plant physiology* 153, no. 2 (2010): 384-395.

He, Jun-Xian, Joshua M. Gendron, Yu Sun, Srinivas SL Gampala, Nathan Gendron, Catherine Qing Sun, and Zhi-Yong Wang. "BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses." *Science* 307, no. 5715 (2005): 1634-1638.

Helaly, Mohamed Naser, Mohamed Ahmed El-Metwally, Hanan El-Hoseiny, Samar Abdelaziz Omar, and Nabil Ibrahim El-Sheery. "Effect of nanoparticles on biological contamination of'in vitro'cultures and organogenic regeneration of banana." *Australian Journal of Crop Science* 8, no. 4 (2014): 612.

He, Kai, Xiaoping Gou, Tong Yuan, Honghui Lin, Tadao Asami, Shigeo Yoshida, Scott D. Russell, and Jia Li. "BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways." *Current Biology* 17, no. 13 (2007): 1109-1115.

Hématy, Kian, Pierre-Etienne Sado, Ageeth Van Tuinen, Soizic Rochange, Thierry Desnos, Sandrine Balzergue, Sandra Pelletier, Jean-Pierre Renou, and Herman Höfte. "A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis." *Current Biology*17, no. 11 (2007): 922-931.

Heise, Andreas, Bernadette Lippok, Christoph Kirsch, and Klaus Hahlbrock. "Two immediate-early pathogen-responsive members of the AtCMPG gene family in Arabidopsis thaliana and the W-box-containing elicitor-response element of AtCMPG1." *Proceedings of the National Academy of Sciences*99, no. 13 (2002): 9049-9054

Hirayama, Takashi, Chikara Ohto, Tsuyoshi Mizoguchi, and Kazuo Shinozaki. "A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in Arabidopsis thaliana." *Proceedings of the National Academy of Sciences* 92, no. 9 (1995): 3903-3907.

Hong, Fashui, Juan Zhou, Chao Liu, Fan Yang, Cheng Wu, Lei Zheng, and Ping Yang. "Effect of nano-TiO 2 on photochemical reaction of chloroplasts of spinach." *Biological trace element research* 105, no. 1-3 (2005): 269-279.

Hu, Wei, Yixing Wang, Christian Bowers, and Hong Ma. "Isolation, sequence analysis, and expression studies of florally expressed cDNAs in Arabidopsis." *Plant molecular biology* 53, no. 4 (2003): 545-563.

Husen, Azamal, and Khwaja Salahuddin Siddiqi. "Carbon and fullerene nanomaterials in plant system." *Journal of Nanobiotechnology* 12, no. 1 (2014): 16.

Huang, Xiaozhen, Lingyan Hou, Jingjing Meng, Huiwen You, Zhen Li, Zhizhong Gong, Shuhua Yang, and Yiting Shi. "The antagonistic action of abscisic acid and cytokinin signaling mediates drought stress response in Arabidopsis." *Molecular plant* 11, no. 7 (2018): 970-982

Hundertmark, Michaela, and Dirk K. Hincha. "LEA (late embryogenesis abundant) proteins and their encoding genes in Arabidopsis thaliana." *BMC genomics* 9, no. 1 (2008): 118.

Hyodo, Hideki, Seiyei Yamakawa, Yuji Takeda, Masao Tsuduki, Akiho Yokota, Kazuhiko Nishitani, and Takayuki Kohchi. "Active gene expression of a xyloglucan endotransglucosylase/hydrolase gene, XTH9, in inflorescence apices is related to cell elongation in Arabidopsis thaliana." *Plant molecular biology* 52, no. 2 (2003): 473-482.

Jaberzadeh, Amir, Payam Moaveni, Hamid Reza TOHIDI MOGHADAM, and Hossein Zahedi. "Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress." *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 41, no. 1 (2013): 201-207.

Jackowski, Grzegorz, Karol Kacprzak, and Stefan Jansson. "Identification of Lhcb1/Lhcb2/Lhcb3 heterotrimers of the main light-harvesting chlorophyll a/b–protein complex of Photosystem II (LHC II)." *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1504, no. 2-3 (2001): 340-345.

Jarvis, Michael C. "Structure and properties of pectin gels in plant cell walls." *Plant, Cell & Environment* 7, no. 3 (1984): 153-164.

Jones, Jonathan DG, and Jeffery L. Dangl. "The plant immune system." *nature* 444, no. 7117 (2006): 323.

Kampranis, Sotirios C., Radostina Damianova, Mirna Atallah, Garabet Toby, Greta Kondi, Philip N. Tsichlis, and Antonios M. Makris. "A novel plant glutathione S-

transferase/peroxidase suppresses Bax lethality in yeast." *Journal of Biological Chemistry* 275, no. 38 (2000): 29207-29216.

Karlova, Rumyana, Sjef Boeren, Eugenia Russinova, José Aker, Jacques Vervoort, and Sacco de Vries. "The Arabidopsis somatic embryogenesis receptor-like kinase1 protein complex includes brassinosteroid-insensitive1." *The Plant Cell* 18, no. 3 (2006): 626-638.

Kaya, Cengiz, and David Higgs. "Response of tomato (Lycopersiconesculentum L.) cultivars to foliar application of Zinc when grown in sand culture at low Zinc." *Scientia Horticulturae* 93, no. 1 (2002): 53-64.

Kaveh, Rashid, Yue-Sheng Li, Sibia Ranjbar, Rouzbeh Tehrani, Christopher L. Brueck, and Benoit Van Aken. "Changes in Arabidopsis thaliana gene expression in response to silver nanoparticles and silver ions." *Environmental science & technology* 47, no. 18 (2013): 10637-10644

Khan, M. Iqbal R., Mehar Fatma, Tasir S. Per, Naser A. Anjum, and Nafees A. Khan. "Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants." *Frontiers in Plant Science* 6 (20 15): 462.

Khanna, Rajnish, Brent Kronmiller, Don R. Maszle, George Coupland, Magnus Holm, Takeshi Mizuno, and Shu-Hsing Wu. "The Arabidopsis B-box zinc finger family." *The Plant Cell* 21, no. 11 (2009): 3416-3420.

Khodakovskaya, Mariya V., Kanishka de Silva, Dmitry A. Nedosekin, Enkeleda Dervishi, Alexandru S. Biris, Evgeny V. Shashkov, Ekaterina I. Galanzha, and Vladimir P. Zharov. "Complex genetic, photothermal, and photoacoustic analysis of nanoparticle-plant interactions." *Proceedings of the National Academy of Sciences* 108, no. 3 (2011): 1028-1033.

Kim, Jae-Hwan, Youngjun Oh, Hakwon Yoon, Inhwan Hwang, and Yoon-Seok Chang. "Iron nanoparticle-induced activation of plasma membrane H+-ATPase promotes stomatal opening in Arabidopsis thaliana." *Environmental science & technology*49, no. 2 (2014): 1113-1119.

Kim, Ji-Hun, Paul G. Tratnyek, and Yoon-Seok Chang. "Rapid dechlorination of polychlorinated dibenzo-p-dioxins by bimetallic and nanosized zerovalent iron." *Environmental science &* technology 42, no. 11 (2008): 4106-4112.

Kim, Tae-Houn, Maik Böhmer, Honghong Hu, Noriyuki Nishimura, and Julian I. Schroeder. "Guard cell signal transduction network: advances in understanding abscisic acid, CO2, and Ca2+ signaling." *Annual review of plant biology* 61 (2010): 561-591.

Kim, Tae-Wuk, Shenheng Guan, Alma L. Burlingame, and Zhi-Yong Wang. "The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2." *Molecular cell* 43, no. 4 (2011): 561-571.

Kim, Tae-Wuk, Shenheng Guan, Yu Sun, Zhiping Deng, Wenqiang Tang, Jian-Xiu Shang, Ying Sun, Alma L. Burlingame, and Zhi-Yong Wang. "Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors." *Nature cell biology* 11, no. 10 (2009): 1254.

Kim, Woe-Yeon, Zahir Ali, Hee Jin Park, Su Jung Park, Joon-Yung Cha, Javier Perez-Hormaeche, Francisco Javier Quintero et al. "Release of SOS2 kinase from sequestration with GIGANTEA determines salt tolerance in Arabidopsis." *Nature communications* 4 (2013): 1352.

Kim, Tae-Wuk, and Zhi-Yong Wang. "Brassinosteroid signal transduction from receptor kinases to transcription factors." *Annual review of plant biology* 61 (2010): 681-704. Ko, Jae-Heung, Seung H. Yang, and Kyung-Hwan Han. "Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis." *The Plant Journal* 47, no. 3 (2006): 343-355.

Kim, Min-Jung, Gah-Hyun Lim, Eun-Seon Kim, Chang-Beom Ko, Kwang-Yeol Yang, Jin-an Jeong, Myung-Chul Lee, and Cheol Soo Kim. "Abiotic and biotic stress tolerance in Arabidopsis overexpressing the multiprotein bridging factor 1a (MBF1a) transcriptional coactivator gene." *Biochemical and biophysical research communications* 354, no. 2 (2007): 440-446.

Kiyosue, Tomohiro, Hiroshi Abe, Kazuko Yamaguchi-Shinozaki, and Kazuo Shinozaki. "ERD6, a cDNA clone for an early dehydration-induced gene of Arabidopsis, encodes a putative sugar transporter." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1370, no. 2 (1998): 187-191.

Knoth, Colleen, and Thomas Eulgem. "The oomycete response gene LURP1 is required for defense against Hyaloperonospora parasitica in Arabidopsis thaliana." *The Plant Journal* 55, no. 1 (2008): 53-64

Kohorn, Bruce D. "Plasma membrane-cell wall contacts." *Plant Physiology* 124, no. 1 (2000): 31-38.

Kohorn, Bruce D., Susan Johansen, Akira Shishido, Tanya Todorova, Rhysly Martinez, Elita Defeo, and Pablo Obregon. "Pectin activation of MAP kinase and gene expression is WAK2 dependent." *The Plant Journal* 60, no. 6 (2009): 974-982.

Kohli, Pooja, Manmohit Kalia, and Reena Gupta. "Pectin methylesterases: a review." *Journal of Bioprocessing & Biotechniques* 5, no. 5 (2015): 1.

Krishna, Priti. "Brassinosteroid-mediated stress responses." *Journal of Plant Growth Regulation* 22, no. 4 (2003): 289-297.

Krishnaraj, C., E. G. Jagan, R. Ramachandran, S. M. Abirami, N. Mohan, and P. T. Kalaichelvan. "Effect of biologically synthesized silver nanoparticles on Bacopa monnieri (Linn.) Wettst. plant growth metabolism." *Process biochemistry* 47, no. 4 (2012): 651-658.

Krishnaswamy, Sowmya, Shiv Verma, Muhammad H. Rahman, and Nat NV Kav. "Functional characterization of four APETALA2-family genes (RAP2. 6, RAP2. 6L, DREB19 and DREB26) in Arabidopsis." *Plant molecular biology* 75, no. 1-2 (2011): 107-127.

Kumar, Vineet, Praveen Guleria, Vinay Kumar, and Sudesh Kumar Yadav. "Gold nanoparticle exposure induces growth and yield enhancement in Arabidopsis thaliana." *Science of the Total Environment* 461 (2013): 462-468.

Klüsener, Birgit, Jared J. Young, Yoshiyuki Murata, Gethyn J. Allen, Izumi C. Mori, Veronique Hugouvieux, and Julian I. Schroeder. "Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in Arabidopsis guard cells." *Plant physiology* 130, no. 4 (2002): 2152-2163

Lattanzio, Vincenzo, Veronica MT Lattanzio, and Angela Cardinali. "Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects." *Phytochemistry: Advances in research* 661, no. 2 (2006): 23-67.

Lamb, Chris, and Richard A. Dixon. "The oxidative burst in plant disease resistance." *Annual review of plant biology* 48, no. 1 (1997): 251-275. crops." *ACS applied materials & interfaces*5, no. 16 (2013): 7965-7973.

Landa, Premysl, Radomira Vankova, Jana Andrlova, Jan Hodek, Petr Marsik, Helena Storchova, Jason C. White, and Tomas Vanek. "Nanoparticle-specific changes in Arabidopsis thaliana gene expression after exposure to ZnO, TiO2, and fullerene soot." *Journal of hazardous materials* 241 (2012): 55-62.

Lee, Sumin, Hyunjung Choi, SuJeoung Suh, In-Suk Doo, Ki-Young Oh, Eun Jeong Choi, Ann T. Schroeder Taylor, Philip S. Low, and Youngsook Lee. "Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and Commelina communis." *Plant physiology* 121, no. 1 (1999): 147-152.

Lee, Sumin, Hyunjung Choi, SuJeoung Suh, In-Suk Doo, Ki-Young Oh, Eun Jeong Choi, Ann T. Schroeder Taylor, Philip S. Low, and Youngsook Lee. "Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and Commelina communis." *Plant physiology* 121, no. 1 (1999): 147-152.

Le Gall, Hyacinthe, Florian Philippe, Jean-Marc Domon, Françoise Gillet, Jérôme Pelloux, and Catherine Rayon. "Cell wall metabolism in response to abiotic stress." *Plants* 4, no. 1 (2015): 112-166.

Lin, Daohui, and Baoshan Xing. "Phytotoxicity of nanoparticles: inhibition of seed germination and root growth." *Environmental Pollution* 150, no. 2 (2007): 243-250.

Li, Junli, Jing Hu, Chuanxin Ma, Yunqiang Wang, Chan Wu, Jin Huang, and Baoshan Xing. "Uptake, translocation and physiological effects of magnetic iron oxide (γ-Fe2O3) nanoparticles in corn (Zea mays L.)." *Chemosphere* 159 (2016): 326-334.

Liepman, Aaron H., Raymond Wightman, Naomi Geshi, Simon R. Turner, and Henrik Vibe Scheller. "Arabidopsis–a powerful model system for plant cell wall research." *The Plant Journal*61, no. 6 (2010): 1107-1121. Li, Jia, Jiangqi Wen, Kevin A. Lease, Jason T. Doke, Frans E. Tax, and John C. Walker. "BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling." *Cell* 110, no. 2 (2002): 213-222.

Lin, Chentao. "Blue light receptors and signal transduction." *The Plant Cell* 14, no. suppl 1 (2002): S207-S225.

Liners, Françoise, Jean-Jacques Letesson, Christian Didembourg, and Pierre Van Cutsem. "Monoclonal antibodies against pectin: recognition of a conformation induced by calcium." *Plant physiology* 91, no. 4 (1989): 1419-1424.

Liu, Tiffany, Jenny Carlsson, Tomomi Takeuchi, Linsey Newton, and Eva M. Farre. "Direct regulation of abiotic responses by the Arabidopsis circadian clock component PRR7." *The plant journal* 76, no. 1 (2013): 101-114.

Liu, Yanan, Xingchuan Huang, Meng Li, Ping He, and Yuelin Zhang. "Loss‐of‐function of Arabidopsis receptor‐like kinase BIR1 activates cell death and defense responses mediated by BAK1 and SOBIR1." *New Phytologist* 212, no. 3 (2016): 637-645

Longoni, Paolo, Damien Douchi, Federica Cariti, Geoffrey Fucile, and Michel Goldschmidt-Clermont. "Phosphorylation of the light-harvesting complex II isoform Lhcb2 is central to state transitions." *Plant physiology* 169, no. 4 (2015): 2874-2883.

Lu, Changmei, Chaoying Zhang, Junqiang Wen, Guorong Wu, and Mingxuan Tao. "Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism." *Soybean Science* 21, no. 3 (2002): 168-171.

Luhua, Song, Sultan Ciftci-Yilmaz, Jeffery Harper, John Cushman, and Ron Mittler. "Enhanced tolerance to oxidative stress in transgenic Arabidopsis plants expressing proteins of unknown function." *Plant physiology* 148, no. 1 (2008): 280-292.

Lu, Hua, Yang Liu, and Jean T. Greenberg. "Structure–function analysis of the plasma membrane‐localized Arabidopsis defense component ACD6." *The Plant Journal* 44, no. 5 (2005): 798-809.

Lu, Dongping, Shujing Wu, Xiquan Gao, Yulan Zhang, Libo Shan, and Ping He. "A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity." *Proceedings of the National Academy of Sciences* 107, no. 1 (2010): 496-501.

Lüdke, Daniel, Charlotte Roth, Denise Hartken, and Marcel Wiermer. "MOS6 and TN13 in plant immunity." *Plant signaling & behavior* 13, no. 4 (2018): 808-821

Lyou, Seoung Hyun, Hyon Jin Park, Choonkyun Jung, Hwang Bae Sohn, Garam Lee, Chung Ho Kim, Minkyun Kim, Yang Do Choi, and Jong-Joo Cheong. "The Arabidopsis AtLEC gene

encoding a lectin-like protein is up-regulated by multiple stimuli including developmental signal, wounding, jasmonate, ethylene, and chitin elicitor." *Molecules and cells* 27, no. 1 (2009): 75-81.

Maynard, Andrew D. "Nanotechnology: the next big thing, or much ado about nothing?" *The Annals of occupational hygiene* 51, no. 1 (2006): 1-12.

Mahmoodzadeh, H., M. Nabavi, and H. Kashefi. "Effect of nanoscale titanium dioxide particles on the germination and growth of canola (Brassica napus)." (2013): 25-32.

Matsushima, Ryo, Maki Kondo, Mikio Nishimura, and Ikuko Hara-Nishimura. "A novel ER-derived compartment, the ER body, selectively accumulates a β-glucosidase with an ER-retention signal in Arabidopsis." *The Plant Journal* 33, no. 3 (2003): 493-502.

Melotto, Maeli, William Underwood, Jessica Koczan, Kinya Nomura, and Sheng Yang He. "Plant stomata function in innate immunity against bacterial invasion." *Cell* 126, no. 5 (2006): 969-980

Mir, Ricardo, M. Luisa Hernández, Eliane Abou-Mansour, José Manuel Martínez-Rivas, Félix Mauch, Jean-Pierre Métraux, and José León. "Pathogen and Circadian Controlled 1 (PCC1) regulates polar lipid content, ABA-related responses, and pathogen defence in *Arabidopsis thaliana*." *Journal of experimental botany* 64, no. 11 (2013): 3385-3395.

Mishina, Tatiana E., and Jürgen Zeier. "Bacterial non-host resistance: interactions of Arabidopsis with non-adapted Pseudomonas syringae strains." *Physiologia plantarum* 131, no. 3 (2007): 448-461.

Mizuno, Takeshi, and Norihito Nakamichi. "Pseudo-response regulators (PRRs) or true oscillator components (TOCs)." *Plant and cell physiology* 46, no. 5 (2005): 677-685.

Mohnen, Debra. "Pectin structure and biosynthesis." *Current opinion in plant biology* 11, no. 3 (2008): 266-277.

Mora-García, Santiago, Grégory Vert, Yanhai Yin, Ana Caño-Delgado, Hyeonsook Cheong, and Joanne Chory. "Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis." *Genes & development* 18, no. 4 (2004): 448-460.

Mukherjee, Arnab, Jose R. Peralta-Videa, Susmita Bandyopadhyay, Cyren M. Rico, Lijuan Zhao, and Jorge L. Gardea-Torresdey. "Physiological effects of nanoparticulate ZnO in green peas (Pisum sativum L.) cultivated in soil." *Metallomics* 6, no. 1 (2014): 132-138.

Nagano, Atsushi J., Yoichiro Fukao, Masayuki Fujiwara, Mikio Nishimura, and Ikuko Hara-Nishimura. "Antagonistic jacalin-related lectins regulate the size of ER body-type β-glucosidase complexes in Arabidopsis thaliana." *Plant and cell physiology* 49, no. 6 (2008): 969-980.

Nakashima, Kazuo, Yasunari Fujita, Norihito Kanamori, Takeshi Katagiri, Taishi Umezawa, Satoshi Kidokoro, Kyonoshin Maruyama et al. "Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2. 2, SRK2E/SnRK2. 6/OST1 and SRK2I/SnRK2. 3, involved in ABA signaling are essential for the control of seed development and dormancy." *Plant and Cell Physiology* 50, no. 7 (2009): 1345-1363.

Nakamichi, Norihito, Miyako Kusano, Atsushi Fukushima, Masanori Kita, Shogo Ito, Takafumi Yamashino, Kazuki Saito, Hitoshi Sakakibara, and Takeshi Mizuno. "Transcript profiling of an Arabidopsis PSEUDO RESPONSE REGULATOR arrhythmic triple mutant reveals a role for the circadian clock in cold stress response." *Plant and Cell Physiology* 50, no. 3 (2009): 447-462

Nam, Kyoung Hee, and Jianming Li. "BRI1/BAK1, a receptor kinase pairs mediating brassinosteroid signaling." *Cell* 110, no. 2 (2002): 203-212.

Narusaka, Yoshihiro, Mari Narusaka, Motoaki Seki, Miki Fujita, Junko Ishida, Maiko Nakashima, Akiko Enju et al. "Expression profiles of Arabidopsis phospholipase A IIA gene in response to biotic and abiotic stresses." *Plant and cell physiology* 44, no. 11 (2003): 1246-1252.

Narusaka, Mari, Motoaki Seki, Taishi Umezawa, Junko Ishida, Maiko Nakajima, Akiko Enju, and Kazuo Shinozaki. "Crosstalk in the responses to abiotic and biotic stresses in Arabidopsis: analysis of gene expression in cytochrome P450 gene superfamily by cDNA microarray." *Plant molecular biology* 55, no. 3 (2004): 327-342.

Nishizawa, Ayako, Yukinori Yabuta, and Shigeru Shigeoka. "Galactinol and raffinose constitute a novel function to protect plants from oxidative damage." *Plant physiology* 147, no. 3 (2008): 1251-1263.

Nishizawa, Ayako, Yukinori Yabuta, and Shigeru Shigeoka. "Galactinol and raffinose constitute a novel function to protect plants from oxidative damage." *Plant physiology* 147, no. 3 (2008): 1251-1263.

Nodzon, Lisa A., Wei-Hui Xu, Yongsheng Wang, Li-Ya Pi, Pranjib K. Chakrabarty, and Wen-Yuan Song. "The ubiquitin ligase XBAT32 regulates lateral root development in Arabidopsis." *The Plant Journal* 40, no. 6 (2004): 996-1006.

Prasad, Ram, Vivek Kumar, and Kumar Suranjit Prasad. "Nanotechnology in sustainable agriculture: present concerns and future aspects." *African Journal of Biotechnology* 13, no. 6 (2014): 705-713.

Pokhrel, Lok R., and Brajesh Dubey. "Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles." *Science of the Total Environment* 452 (2013): 321-332.

Prasad, T. N. V. K. V., P. Sudhakar, Y. Sreenivasulu, P. Latha, V. Munaswamy, K. Raja Reddy, T. S. Sreeprasad, P. R. Sajanlal, and T. Pradeep. "Effect of nanoscale zinc oxide particles on

the germination, growth and yield of peanut." *Journal of plant nutrition* 35, no. 6 (2012): 905-927.

Prasad, Bishun D., Shilpi Goel, and Priti Krishna. "In silico identification of carboxylate clamp type tetratricopeptide repeat proteins in Arabidopsis and rice as putative co-chaperones of Hsp90/Hsp70." *PloS one* 5, no. 9 (2010): e12761.

Qi, Yiping, Kenichi Tsuda, Le V. Nguyen, Xia Wang, Jinshan Lin, Angus S. Murphy, Jane Glazebrook, Hans Thordal-Christensen, and Fumiaki Katagiri. "Physical association of Arabidopsis hypersensitive induced reaction proteins (HIRs) with the immune receptor RPS2." *Journal of Biological Chemistry* 286, no. 36 (2011): 31297-31307

Qu, Shaofeng, Xi Zhang, Yutong Song, Jinxing Lin, and Xiaoyi Shan. "THESEUS1 positively modulates plant defense responses against Botrytis cinerea through GUANINE EXCHANGE FACTOR4 signaling." *Journal of integrative plant biology* 59, no. 11 (2017): 797-804.

Qu, Tao, Rongfang Liu, Wei Wang, Lizhe An, Tuo Chen, Guangxiu Liu, and Zhiguang Zhao. "Brassinosteroids regulate pectin methylesterase activity and AtPME41 expression in Arabidopsis under chilling stress." *Cryobiology* 63, no. 2 (2011): 111-117.

Qu, Tao, Rongfang Liu, Wei Wang, Lizhe An, Tuo Chen, Guangxiu Liu, and Zhiguang Zhao. "Brassinosteroids regulate pectin methylesterase activity and AtPME41 expression in Arabidopsis under chilling stress." *Cryobiology* 63, no. 2 (2011): 111-117.

Raliya, Ramesh, and Jagadish Chandra Tarafdar. "ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in Clusterbean (Cyamopsis tetragonoloba L.)." *Agricultural Research* 2, no. 1 (2013): 48-57.

Ramonell, Katrina, Marta Berrocal-Lobo, Serry Koh, Jinrong Wan, Herb Edwards, Gary Stacey, and Shauna Somerville. "Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen Erysiphe cichoracearum." *Plant physiology* 138, no. 2 (2005): 1027-1036.

Ramesh, Madhumitha, Karthikka Palanisamy, K. Babu, and Navin Kumar Sharma. "Effects of bulk & nano-titanium dioxide and zinc oxide on physio-morphological changes in Triticum aestivum Linn." *J Glob Biosci* 3 (2014): 415-422.

Raskar, S. V., and S. L. Laware. "Effect of zinc oxide nanoparticles on cytology and seed germination in onion." *Int J Curr Microbiol App Sci* 3, no. 2 (2014): 467

Ré, Delfina A., Matías Capella, Gustavo Bonaventure, and Raquel L. Chan. "Arabidopsis AtHB7 and AtHB12 evolved divergently to fine tune processes associated with growth and responses to water stress." *BMC plant biology* 14, no. 1 (2014): 150.

Reyes, José C., M. Isabel Muro-Pastor, and Francisco J. Florencio. "The GATA family of transcription factors in Arabidopsis and rice." *Plant physiology* 134, no. 4 (2004): 1718-1732.

Riechmann, Jose Luis, and Elliot M. Meyerowitz. "The AP2/EREBP family of plant transcription factors." *Biological chemistry* 379 (1998): 633-646

Robatzek, Silke, and Imre E. Somssich. "Targets of AtWRKY6 regulation during plant senescence and pathogen defense." *Genes & development* 16, no. 9 (2002): 1139-1149.

Roco, Mihail C. "Environmentally responsible development of nanotechnology." (2005): 106A-112A.

Rosado, Abel, Arnaldo L. Schapire, Ray A. Bressan, Antoine L. Harfouche, Paul M. Hasegawa, Victoriano Valpuesta, and Miguel A. Botella. "The Arabidopsis tetratricopeptide repeat-containing protein TTL1 is required for osmotic stress responses and abscisic acid sensitivity." *Plant Physiology* 142, no. 3 (2006): 1113-1126.

Roux, Milena, Benjamin Schwessinger, Catherine Albrecht, Delphine Chinchilla, Alexandra Jones, Nick Holton, Frederikke Gro Malinovsky, Mahmut Tör, Sacco de Vries, and Cyril Zipfel. "The Arabidopsis leucine-rich repeat receptor–like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens." *The Plant Cell* 23, no. 6 (2011): 2440-2455.

Rushton, Paul J., Imre E. Somssich, Patricia Ringler, and Qingxi J. Shen. "WRKY transcription factors." *Trends in plant science* 15, no. 5 (2010): 247-258.

Sakuma, Yoh, Qiang Liu, Joseph G. Dubouzet, Hiroshi Abe, Kazuo Shinozaki, and Kazuko Yamaguchi-Shinozaki. "DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration-and cold-inducible gene expression." *Biochemical and biophysical research communications* 290, no. 3 (2002): 998-1009.

Salata, Oleg V. "Applications of nanoparticles in biology and medicine." *Journal of nanobiotechnology* 2, no. 1 (2004): 3.

Sauerbrunn, Nicolas, and Nikolaus L. Schlaich. "PCC1: a merging point for pathogen defence and circadian signalling in Arabidopsis." *Planta* 218, no. 4 (2004): 552-561.

Sedghi, Mohammad, Mitra Hadi, and Sahar Gholi Toluie. "Effect of nano zinc oxide on the germination parameters of soybean seeds under drought stress." *Annales of West University of Timisoara. Series of Biology* 16, no. 2 (2013): 73.

Seok, Hye-Yeon, Dong-Hyuk Woo, Linh Vu Nguyen, Huong T. Tran, Vaishali N. Tarte, Syed Muhammad Muntazir Mehdi, Sun-Young Lee, and Yong-Hwan Moon. "Arabidopsis AtNAP functions as a negative regulator via repression of AREB1 in salt stress response." *Planta* 245, no. 2 (2017): 329-341.

Serrano, Mario, Bangjun Wang, Bibek Aryal, Christophe Garcion, Eliane Abou-Mansour, Silvia Heck, Markus Geisler, Felix Mauch, Christiane Nawrath, and Jean-Pierre Métraux. "Export of salicylic acid from the chloroplast requires the multidrug and toxin extrusion-like transporter EDS5." *Plant physiology* 162, no. 4 (2013): 1815-1821

Schenk, Peer M., Kemal Kazan, Anca G. Rusu, John M. Manners, and Donald J. Maclean. "The SEN1 gene of Arabidopsis is regulated by signals that link plant defence responses and senescence." *Plant Physiology and Biochemistry* 43, no. 10-11 (2005): 997-1005.

Schulze, Birgit, Tobias Mentzel, Anna K. Jehle, Katharina Mueller, Seraina Beeler, Thomas Boller, Georg Felix, and Delphine Chinchilla. "Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1." *Journal of Biological Chemistry* 285, no. 13 (2010): 9444-9451.

Schön, Moritz, Armin Töller, Celia Diezel, Charlotte Roth, Lore Westphal, Marcel Wiermer, and Imre E. Somssich. "Analyses of wrky18 wrky40 plants reveal critical roles of SA/EDS1 signaling and indole-glucosinolate biosynthesis for Golovinomyces orontii resistance and a loss-of resistance towards Pseudomonas syringae pv. tomato AvrRPS4." *Molecular Plant-Microbe Interactions* 26, no. 7 (2013): 758-767.

Schröder, Florian, Janina Lisso, Peggy Lange, and Carsten Müssig. "The extracellular EXO protein mediates cell expansion in Arabidopsis leaves." *BMC plant biology* 9, no. 1 (2009): 20.

Schuler, Mary A. "Plant cytochrome P450 monooxygenases." *Critical reviews in plant sciences* 15, no. 3 (1996): 235-284

Sharon, Nathan, and Halina Lis. "How proteins bind carbohydrates: lessons from legume lectins." *Journal of agricultural and food chemistry* 50, no. 22 (2002): 6586-6591.

Shen, Cong-Xiang, Quan-Fang Zhang, Jian Li, Fang-Cheng Bi, and Nan Yao. "Induction of programmed cell death in Arabidopsis and rice by single-wall carbon nanotubes." *American Journal of Botany* 97, no. 10 (2010): 1602-1609.

Shen, Qian-Hua, Yusuke Saijo, Stefan Mauch, Christoph Biskup, Stéphane Bieri, Beat Keller, Hikaru Seki, Bekir Ülker, Imre E. Somssich, and Paul Schulze-Lefert. "Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses." *science* 315, no. 5815 (2007): 1098-1103.

Shi, Hua, Qiujing Shen, Yiping Qi, Haojie Yan, Haozhen Nie, Yongfang Chen, Ting Zhao, Fumiaki Katagiri, and Dingzhong Tang. "BR-SIGNALING KINASE1 physically associates with FLAGELLIN SENSING2 and regulates plant innate immunity in Arabidopsis." *The Plant Cell* 25, no. 3 (2013): 1143-1157.

Singh, Archana, Gah-Hyun Lim, and Pradeep Kachroo. "Transport of chemical signals in systemic acquired resistance." *Journal of integrative plant biology* 59, no. 5 (2017): 336-344

Smith, Aaron P., Ben P. DeRidder, Woei-Jiun Guo, Erin H. Seeley, Fred E. Regnier, and Peter B. Goldsbrough. "Proteomic analysis of Arabidopsis glutathione S-transferases from benoxacor-and copper-treated seedlings." *Journal of Biological Chemistry* 279, no. 25 (2004): 26098-26104.

Song, Young Hun, Daniel A. Estrada, Richard S. Johnson, Somi K. Kim, Sang Yeol Lee, Michael J. MacCoss, and Takato Imaizumi. "Distinct roles of FKF1, GIGANTEA, and ZEITLUPE proteins in the regulation of CONSTANS stability in Arabidopsis photoperiodic flowering." *Proceedings of the National Academy of Sciences* 111, no. 49 (2014): 17672-17677.

Sothern, Robert B., Tong-Seung Tseng, Sandra L. Orcutt, Neil E. Olszewski, and Willard L. Koukkari. "GIGANTEA and SPINDLY genes linked to the clock pathway that controls circadian characteristics of transpiration in Arabidopsis." *Chronobiology international* 19, no. 6 (2002): 1005-1022.

Stacey, Gary, and Naoto Shibuya. "Chitin recognition in rice and legumes." *Plant and Soil* 194, no. 1-2 (1997): 161-169.

Srinivasan, C., and R. Saraswathi. "Nano-agriculture—carbon nanotubes enhance tomato seed germination and plant growth." *Current science* 99, no. 3 (2010): 274-275.

Srivastava, Nupur, Vijay K. Gonugunta, Mallikarjuna R. Puli, and Agepati S. Raghavendra. "Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of Pisum sativum." *Planta* 229, no. 4 (2009): 757-765.

Stampoulis, Dimitrios, Saion K. Sinha, and Jason C. White. "Assay-dependent phytotoxicity of nanoparticles to plants." *Environmental Science & Technology* 43, no. 24 (2009): 9473-9479.

Subbaiah, Layam Venkata, Tollamadugu Naga Venkata Krishna Vara Prasad, Thimmavajjula Giridhara Krishna, Palagiri Sudhakar, Balam Ravindra Reddy, and Thalappil Pradeep. "Novel effects of nanoparticulate delivery of zinc on growth, productivity, and zinc biofortification in maize (Zea mays L.)." *Journal of agricultural and food chemistry* 64, no. 19 (2016): 3778-3788.

Sun, Yu, Xi-Ying Fan, Dong-Mei Cao, Wenqiang Tang, Kun He, Jia-Ying Zhu, Jun-Xian He et al. "Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis." *Developmental cell* 19, no. 5 (2010): 765-777

Suzuki, Nobuhiro, Ludmila Rizhsky, Hongjian Liang, Joel Shuman, Vladimir Shulaev, and Ron Mittler. "Enhanced tolerance to environmental stress in transgenic plants expressing the transcriptional coactivator multiprotein bridging factor 1c." *Plant Physiology* 139, no. 3 (2005): 1313-1322.

Sun, Yu, Xi-Ying Fan, Dong-Mei Cao, Wenqiang Tang, Kun He, Jia-Ying Zhu, Jun-Xian He et al. "Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis." *Developmental cell* 19, no. 5 (2010): 765-777.

Syu, You-yu, Jui-Hung Hung, Jui-Chang Chen, and Huey-wen Chuang. "Impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression." *Plant physiology and biochemistry* 83 (2014): 57-64.

Szymanski, Maciej, Miros³awa Z. Barciszewska, Marek Zywicki, and Jan Barciszewski. "Noncoding RNA transcripts." *Journal of applied genetics* 44, no. 1 (2003): 1-20.

Tang, Wenqiang, Tae-Wuk Kim, Juan A. Oses-Prieto, Yu Sun, Zhiping Deng, Shengwei Zhu, Ruiju Wang, Alma L. Burlingame, and Zhi-Yong Wang. "BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis." *Science* 321, no. 5888 (2008): 557-560.

Takayama, Seiji, Hiroshi Shiba, Megumi Iwano, Kosuke Asano, Minoru Hara, Fang-Sik Che, Masao Watanabe, Kokichi Hinata, and Akira Isogai. "Isolation and characterization of pollen coat proteins of Brassica campestris that interact with S locus-related glycoprotein 1 involved in pollen–stigma adhesion." *Proceedings of the National Academy of Sciences* 97, no. 7 (2000): 3765-3770.

Torabian, Shahram, Morteza Zahedi, and Amir Hossein Khoshgoftar. "Effects of foliar spray of two kinds of zinc oxide on the growth and ion concentration of sunflower cultivars under salt stress." *Journal of plant nutrition* 39, no. 2 (2016): 172-180.

Tang, Yaxiong, Anping Chen, Shigui Liu, and Guixian Xia. "Identification and characterization of a salt tolerance-responsive gene (AtGRP9) of Arabidopsis." *Progress in Natural Science* 13, no. 1 (2003): 50-54.

Tateda, Chika, Zhongqin Zhang, Jay Shrestha, Joanna Jelenska, Delphine Chinchilla, and Jean T. Greenberg. "Salicylic acid regulates Arabidopsis microbial pattern receptor kinase levels and signaling." *The plant cell* 26, no. 10 (2014): 4171-4187.

Tateda, Chika, Zhongqin Zhang, and Jean T. Greenberg. "Linking pattern recognition and salicylic acid responses in Arabidopsis through accelerated cell death6 and receptors." *Plant signaling & behavior* 10, no. 10 (2015): e1010912.

Tojo, Takuto, Kenichi Tsuda, Takeshi Yoshizumi, Akira Ikeda, Junji Yamaguchi, Minami Matsui, and Ken-ichi Yamazaki. "Arabidopsis MBF1s control leaf cell cycle and its expansion." *Plant and cell physiology* 50, no. 2 (2008): 254-264.

Tsuda, Kenichi, and Imre E. Somssich. "Transcriptional networks in plant immunity." *New Phytologist* 206, no. 3 (2015): 932-947.

Valdés, Ana Elisa, Elin Övernäs, Henrik Johansson, Alvaro Rada-Iglesias, and Peter Engström. "The homeodomain-leucine zipper (HD-Zip) class I transcription factors ATHB7 and ATHB12 modulate abscisic acid signalling by regulating protein phosphatase 2C and abscisic acid receptor gene activities." *Plant molecular biology* 80, no. 4-5 (2012): 405-418.

Van der Does, Dieuwertje, Freddy Boutrot, Timo Engelsdorf, Jack Rhodes, Joseph F. McKenna, Samantha Vernhettes, Iko Koevoets et al. "The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses." *PLoS genetics* 13, no. 6 (2017): e1006832

Villagarcia, Hector, Enkeleda Dervishi, Kanishka de Silva, Alexandru S. Biris, and Mariya V. Khodakovskaya. "Surface chemistry of carbon nanotubes impacts the growth and expression of water channel protein in tomato plants." *Small* 8, no. 15 (2012): 2328-2334

Wagner, Ulrich, Robert Edwards, David P. Dixon, and Felix Mauch. "Probing the diversity of the Arabidopsis glutathione S-transferase gene family." *Plant molecular biology* 49, no. 5 (2002): 515-532.

Wang, Haijiao, Cangjin Yang, Chi Zhang, Niyan Wang, Dihong Lu, Jie Wang, Shanshan Zhang, Zhi-Xin Wang, Hong Ma, and Xuelu Wang. "Dual role of BKI1 and 14-3-3 s in brassinosteroid signaling to link receptor with transcription factors." *Developmental cell* 21, no. 5 (2011): 825-834.

Wang, Jing, Yeonjong Koo, Anne Alexander, Yu Yang, Samantha Westerhof, Qingbo Zhang, Jerald L. Schnoor, Vicki L. Colvin, Janet Braam, and Pedro JJ Alvarez. "Phytostimulation of poplars and Arabidopsis exposed to silver nanoparticles and Ag+ at sublethal concentrations." *Environmental science & technology* 47, no. 10 (2013): 5442-54

Wang, Xiao-Yan, Dian-Zhen Li, Qi Li, Yan-Qin Ma, Jing-Wen Yao, Xuan Huang, and Zi-Qin Xu. "Metabolomic analysis reveals the relationship between AZI1 and sugar signaling in systemic acquired resistance of Arabidopsis." *Plant physiology and biochemistry* 107 (2016): 273-287. Wang, Zhi-Yong. "Brassinosteroids modulate plant immunity at multiple levels." *Proceedings of the National Academy of Sciences* 109, no. 1 (2012): 7-8.

Watson, Jean-Luc, Tommy Fang, Christian O. Dimkpa, David W. Britt, Joan E. McLean, Astrid Jacobson, and Anne J. Anderson. "The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties." *Biometals* 28, no. 1 (2015): 101-112.

Wong, Chui E., Yong Li, Aurelie Labbe, David Guevara, Paulo Nuin, Brett Whitty, Claudia Diaz et al. "Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in Thellungiella, a close relative of Arabidopsis." *Plant Physiology* 140, no. 4 (2006): 1437-1450.

Wrzaczek, Michael, Mikael Brosché, Jarkko Salojärvi, Saijaliisa Kangasjärvi, Niina Idänheimo, Sophia Mersmann, Silke Robatzek, Stanisław Karpiński, Barbara Karpińska, and Jaakko Kangasjärvi. "Transcriptional regulation of the CRK/DUF26 group of receptor-like protein kinases by ozone and plant hormones in Arabidopsis." *BMC plant biology* 10, no. 1 (2010): 95.

Xu, Yan-Hong, Rui Liu, Lu Yan, Zhi-Qiang Liu, Shang-Chuan Jiang, Yuan-Yue Shen, Xiao-Fang Wang, and Da-Peng Zhang. "Light-harvesting chlorophyll a/b-binding proteins are required for stomatal response to abscisic acid in Arabidopsis." *Journal of experimental botany* 63, no. 3 (2011): 1095-1106.

Yamada, Kohji, Yusuke Saijo, Hirofumi Nakagami, and Yoshitaka Takano. "Regulation of sugar transporter activity for antibacterial defense in Arabidopsis." *Science* 354, no. 6318 (2016): 1427-1430.

Yamaguchi-Shinozakiaib, Kazuko, and Kazuo Shinozaki. "A Nove1 cis-Acting Element in an Arabidopsis Gene 1s lnvolved in Responsiveness to Drought, Lowqemperature, or High-Salt Stress."

Yanhui, Chen, Yang Xiaoyuan, He Kun, Liu Meihua, Li Jigang, Gao Zhaofeng, Lin Zhiqiang et al. "The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family." *Plant molecular biology* 60, no. 1 (2006): 107-124.

Yang, Ling, and Daniel J. Watts. "Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles." *Toxicology letters* 158, no. 2 (2005): 122-132.

Yang, Fan, Fashui Hong, Wenjuan You, Chao Liu, Fengqing Gao, Cheng Wu, and Ping Yang. "Influence of nano-anatase TiO 2 on the nitrogen metabolism of growing spinach." *Biological trace element research* 110, no. 2 (2006): 179-190.

Yang, Yuanai, Yaxi Zhang, Pingtao Ding, Kaeli Johnson, Xin Li, and Yuelin Zhang. "The ankyrin-repeat transmembrane protein BDA1 functions downstream of the receptor-like protein SNC2 to regulate plant immunity." *Plant physiology*159, no. 4 (2012): 1857-1865.

Yang, Tianbao, and B. W. Poovaiah. "Calcium/calmodulin-mediated signal network in plants." *Trends in plant science* 8, no. 10 (2003): 505-512.

Yeung, Elaine, Hans van Veen, Divya Vashisht, Ana Luiza Sobral Paiva, Maureen Hummel, Tom Rankenberg, Bianka Steffens et al. "A stress recovery signaling network for enhanced flooding tolerance in Arabidopsis thaliana." *Proceedings of the National Academy of Sciences* 115, no. 26 (2018): E6085-E6094.

Yoon, Sung-Ji, Jin Il Kwak, Woo-Mi Lee, Patricia A. Holden, and Youn-Joo An. "Zinc oxide nanoparticles delay soybean development: a standard soil microcosm study." *Ecotoxicology and environmental safety* 100 (2014): 131-137.

Yu, Keshun, Juliana Moreira Soares, Mihir Kumar Mandal, Caixia Wang, Bidisha Chanda, Andrew N. Gifford, Joanna S. Fowler, Duroy Navarre, Aardra Kachroo, and Pradeep Kachroo. "A feedback regulatory loop between G3P and lipid transfer proteins DIR1 and AZI1 mediates azelaic-acid-induced systemic immunity." *Cell Reports* 3, no. 4 (2013): 1266-1278.

Yu, Xiaofei, Lei Li, Jaroslaw Zola, Maneesha Aluru, Huaxun Ye, Andrew Foudree, Hongqing Guo et al. "A brassinosteroid transcriptional network revealed by genome‐wide identification of BESI target genes in Arabidopsis thaliana." *The Plant Journal* 65, no. 4 (2011): 634-646.

Ze, Yuguan, Chao Liu, Ling Wang, Mengmeng Hong, and Fashui Hong. "The regulation of TiO 2 nanoparticles on the expression of light-harvesting complex II and photosynthesis of chloroplasts of Arabidopsis thaliana." *Biological trace element research* 143, no. 2 (2011): 1131-1141.

Zhang, Jihong, Xiushan Li, Zhimin He, Xiaoying Zhao, Qiming Wang, Bo Zhou, Dashi Yu et al. "Molecular character of a phosphatase 2C (PP2C) gene relation to stress tolerance in Arabidopsis thaliana." *Molecular biology reports* 40, no. 3 (2013): 2633-2644

Zhang, Ruichang, Haibo Zhang, Chen Tu, Xuefeng Hu, Lianzhen Li, Yongming Luo, and Peter Christie. "Phytotoxicity of ZnO nanoparticles and the released Zn (II) ion to corn (Zea mays L.) and cucumber (Cucumis sativus L.) during germination." *Environmental Science and Pollution Research*22, no. 14 (2015): 11109-11117.

Zhou, Yu-Ping, Jun Duan, Takahiro Fujibe, Kotaro T. Yamamoto, and Chang-En Tian. "AtIQM1, a novel calmodulin-binding protein, is involved in stomatal movement in Arabidopsis." *Plant molecular biology* 79, no. 4-5 (2012): 333-346

Zipfel, Cyril. "Pattern-recognition receptors in plant innate immunity." *Current opinion in immunology* 20, no. 1 (2008): 10-16. Zheng, Zuyu, Stephen L. Mosher, Baofang Fan, Daniel F. Klessig, and Zhixiang Chen. "Functional analysis of Arabidopsis WRKY25 transcription factor in plant defense against Pseudomonas syringae." *BMC plant biology* 7, no. 1 (2007): 2.

APPENDIX A. TRANSCRIPTS DOWNREGULATED IN RESPONSE TO ZNONP TREATED PLANTS COMPARED TO UNTREATED CONTROL PLANTS AND ZN^{+2} ION CONTROL PLANTS

Feature ID	Ctrlvs10_FC	Ctrlvs10_FDR	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT3G01345	-44.78	< 0.05	120.99	< 0.05	141	3	0	2	0	463	0	1	1
AT4G37370	-7.55	< 0.05	4.63	< 0.05	178	13	39	42	65	48	9	11	15
AT5G52750	-10.35	< 0.05	9.36	< 0.05	48	2	20	20	27	22	3	0	4
AT2G26560	-7.74	< 0.05	7.85	< 0.05	71	6	33	45	33	42	7	1	7
AT3G16530	-3.74	< 0.05	4.02	< 0.05	86	31	65	60	91	55	23	20	12
ATCG00960	-7.92	< 0.05	9.04	< 0.05	2	4	2	6	1	3	0	1	0
AT4G12470	-3.01	< 0.05	4.41	< 0.05	112	49	96	252	101	44	20	24	51
AT2G05440	-3.53	< 0.05	5.26	< 0.05	59	20	93	110	67	89	11	13	28

* FC: fold change *Ctrl-R: R eads in untreated control plants *19.7-R: Reads in Zn +2 ion treated control plants *10-R: Reads in ZnONPs treated plants

APPENDIX B. TRANSCRIPTS DOWNREGULATED IN THE ZNONP TREATED PLANTS COMPARED TO THE UNTREATED CONTROL PLANTS

Feature ID	Ctrlvs10_FC	Ctrlvs10_FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT2G03760	-3.43	< 0.05	245	15	39	39	53	31	36	34	33
AT4G12490	-3.12	< 0.05	50	53	184	71	19	19	33	4	62
AT2G39330	-3.46	< 0.05	164	201	156	38	89	95	54	38	81
AT3G05727	-3.36	< 0.05	17	92	12	9	54	0	8	29	4
AT1G02930	-3.63	< 0.05	88	41	101	91	74	25	6	22	40
AT2G47000	-7.43	< 0.05	216	23	40	16	13	29	13	16	16
AT1G72260	-10.53	< 0.05	22	39	30	6	11	19	4	0	5
AT3G16670	-4.01	< 0.05	37	20	32	15	13	11	12	10	3
AT2G20870	-3.24	< 0.05	11	94	21	22	6	35	7	2	35
ATCG00010	-3.34	< 0.05	4	5	0	5	0	0	0	3	0

APPENDIX C. TRANSCRIPTS DOWNREGULATED IN THE ZNONP TREATED PLANTS COMPARED TO THE ZN^{+2} ION TREATED CONTROL PLANTS

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT2G41100	11.33	< 0.05	449	60	415	1581	2212	770	75	241	120
AT5G52882	3.58	< 0.05	91	96	230	453	400	442	174	83	135
AT1G59710	3.96	< 0.05	49	51	87	181	205	179	51	51	51
AT3G45860	3.14	< 0.05	32	21	58	122	109	102	37	59	22
AT2G23130	4.77	< 0.05	19	60	102	292	155	118	28	74	21
AT4G28490	3.65	< 0.05	26	39	48	110	98	171	45	35	28
AT3G59080	3.78	< 0.05	12	18	37	87	67	65	20	20	20
AT4G35320	4.25	< 0.05	19	31	68	135	145	96	24	46	23
AT3G17390	4.12	< 0.05	700	906	1190	3751	3232	2168	774	894	718
AT2G13790	4.17	< 0.05	58	51	107	234	272	230	85	44	57
AT4G02540	3.91	< 0.05	53	81	201	401	382	309	86	115	94
AT4G21870	3.15	< 0.05	28	23	34	128	81	81	42	37	18
AT1G68410	3.17	< 0.05	41	39	68	169	201	127	61	48	60
AT4G13505	3.55	< 0.05	125	125	68	521	219	390	85	154	91
AT1G79700	3.93	< 0.05	48	57	75	235	240	139	73	70	42
AT5G14450	3.40	< 0.05	35	38	80	191	166	129	47	70	35
AT4G20780	4.76	< 0.05	9	9	18	46	34	44	11	13	3
AT3G50650	4.81	< 0.05	17	26	66	134	110	122	25	27	27
AT4G28190	3.77	< 0.05	26	31	54	162	133	88	28	52	25
AT5G40450	4.26	< 0.05	457	506	731	2344	1453	2052	499	448	506
AT1G27460	3.45	< 0.05	54	54	133	262	338	206	78	73	99
AT2G17500	3.73	< 0.05	28	19	35	84	121	85	26	25	30
AT1G22530	4.73	< 0.05	199	287	608	1379	1117	1168	238	365	219
AT1G04430	3.50	< 0.05	211	257	311	1064	873	782	279	294	258
AT1G72790	3.57	< 0.05	33	46	39	204	118	100	31	44	48
AT1G20970	3.48	< 0.05	104	128	161	586	423	375	140	114	170
AT1G17620	4.45	< 0.05	52	64	98	300	226	222	41	65	70

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT5G51460	3.47	< 0.05	28	32	92	174	192	152	59	59	44
AT1G75310	3.82	< 0.05	45	58	113	295	242	226	70	60	80
AT2G36410	3.44	< 0.05	40	76	111	264	312	210	92	80	74
AT5G35750	3.04	< 0.05	69	58	169	406	307	329	154	94	121
AT2G28130	4.34	< 0.05	15	13	35	66	98	47	22	12	22
AT1G76520	3.89	< 0.05	67	40	113	287	270	234	68	66	82
AT3G25500	4.32	< 0.05	78	100	213	554	361	492	118	109	116
AT1G63260	3.38	< 0.05	32	45	85	193	219	165	70	51	63
AT1G22882	4.79	< 0.05	45	58	127	326	278	216	65	53	64
AT5G09440	4.31	< 0.05	109	90	133	504	397	311	50	137	106
AT1G53430	4.35	< 0.05	64	58	162	329	363	308	94	67	82
AT2G31880	5.71	< 0.05	78	53	111	251	355	273	57	43	64
AT3G05490	4.09	< 0.05	81	81	143	423	467	209	73	149	63
AT1G66180	3.80	< 0.05	51	48	94	299	233	173	71	76	51
AT5G01810	3.72	< 0.05	71	104	233	586	473	407	111	176	128
AT2G34510	4.51	< 0.05	124	163	259	875	660	523	160	193	133
AT2G42040	3.66	< 0.05	35	52	87	201	227	171	55	69	55
AT2G48030	5.84	< 0.05	24	34	63	194	131	141	23	28	31
AT5G54380	4.24	< 0.05	107	202	399	1021	879	756	159	317	181
AT5G03520	3.87	< 0.05	120	91	203	611	598	361	136	165	136
AT2G06850	3.98	< 0.05	695	875	1279	3636	3847	3307	842	1158	930
AT1G22740	4.15	< 0.05	37	40	99	203	271	179	63	64	49
AT5G08150	3.95	< 0.05	6	6	16	40	45	24	4	21	3
AT1G35350	6.06	< 0.05	22	16	54	139	138	85	20	18	26
AT1G07000	7.42	< 0.05	25	15	34	102	84	114	23	6	13
AT3G62720	4.80	< 0.05	41	57	104	304	300	177	42	80	49
AT5G62920	8.13	< 0.05	23	22	72	117	173	181	17	17	24

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT5G20250	3.53	< 0.05	1143	1495	1874	8834	6027	2682	1315	2082	1948
AT1G76680	5.33	< 0.05	110	78	107	496	495	175	56	86	91
AT4G33950	4.10	< 0.05	30	43	78	209	241	136	51	41	61
AT2G30930	4.25	< 0.05	158	188	335	833	984	804	186	302	177
AT4G32800	6.89	< 0.05	10	14	15	70	71	20	8	8	8
AT1G76160	4.83	< 0.05	243	321	451	1757	1385	917	317	290	301
AT5G66920	3.79	< 0.05	45	58	68	276	172	269	62	64	73
AT3G47570	4.20	< 0.05	28	19	62	143	170	136	44	30	39
AT1G08930	5.95	< 0.05	268	150	364	1221	1151	770	141	263	158
AT1G22400	4.40	< 0.05	33	29	42	216	160	68	41	33	37
AT3G57520	3.70	< 0.05	722	957	1345	5956	4357	1933	847	1275	1470
AT3G62150	7.29	< 0.05	41	18	46	186	127	148	20	16	27
AT1G03870	4.99	< 0.05	174	341	383	1694	1051	1061	177	419	196
AT5G65390	3.65	< 0.05	40	50	47	266	126	206	30	84	54
AT4G08930	3.63	< 0.05	17	23	64	147	182	82	32	40	50
AT4G33420	3.32	< 0.05	7	17	15	89	57	28	15	19	21
AT2G31730	4.88	< 0.05	9	15	30	96	84	41	14	15	17
AT1G20780	4.89	< 0.05	41	44	73	255	200	234	64	38	48
AT1G35710	6.80	< 0.05	45	37	79	160	159	400	52	28	29
AT1G76090	4.13	< 0.05	44	62	73	369	250	163	52	85	63
AT4G35770	3.19	< 0.05	373	317	403	1857	1856	1034	340	682	568
AT3G04210	8.96	< 0.05	57	29	100	209	315	291	27	49	19
AT1G17990	6.82	< 0.05	41	22	117	176	463	148	41	39	43
AT5G22500	3.95	< 0.05	35	35	28	185	181	84	56	39	30
AT5G05440	6.34	< 0.05	47	38	96	383	311	86	28	61	41
AT2G34770	4.55	< 0.05	96	130	321	798	871	556	171	222	139
AT5G65470	3.61	< 0.05	56	46	93	358	339	172	85	91	80

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT1G21250	8.51	< 0.05	121	83	167	419	609	646	98	61	52
AT2G38310	7.81	< 0.05	55	57	57	371	186	234	28	51	26
AT5G42250	8.21	< 0.05	16	8	20	57	81	64	11	5	8
AT1G19050	3.62	< 0.05	24	22	49	114	144	186	29	53	46
AT5G20230	9.80	< 0.05	65	21	72	286	329	87	10	23	44
AT5G23750	4.13	< 0.05	19	22	37	130	104	131	36	29	29
AT1G61100	5.44	< 0.05	112	171	468	1257	1329	751	240	228	193
AT4G13340	5.83	< 0.05	66	146	210	999	442	529	65	217	65
AT3G59350	5.17	< 0.05	176	137	327	965	1298	690	212	202	203
AT5G39580	16.31	< 0.05	14	14	47	156	168	15	5	6	10
AT2G44500	4.93	< 0.05	28	60	110	397	332	187	45	89	61
AT4G14400	7.23	< 0.05	368	235	295	1028	1084	2433	378	152	151
AT2G31010	5.67	< 0.05	19	14	40	145	114	104	19	20	26
AT2G42580	6.02	< 0.05	107	147	187	859	738	558	120	133	128
AT3G23730	6.23	< 0.05	56	91	90	496	406	275	50	92	61
AT5G65610	4.42	< 0.05	16	24	56	121	144	102	27	42	22
AT5G06530	3.35	< 0.05	33	32	71	222	227	236	68	68	79
AT1G72416	4.52	< 0.05	21	22	59	195	207	84	47	48	20
AT2G24600	16.01	< 0.05	20	7	51	126	152	107	6	13	4
AT2G46440	9.46	< 0.05	41	17	48	109	167	249	29	17	11
AT2G33570	5.66	< 0.05	36	48	92	375	285	211	43	70	48
AT2G16660	4.44	< 0.05	37	44	32	336	154	105	41	26	73
AT5G03355	6.31	< 0.05	10	8	23	84	67	62	5	22	6
AT1G52290	6.61	< 0.05	21	14	44	140	118	143	27	17	19
AT2G14560	15.67	< 0.05	128	36	52	241	184	763	52	19	8
AT1G57990	7.57	< 0.05	49	34	64	287	350	114	28	53	24
AT2G37025	4.70	< 0.05	8	6	33	67	79	78	19	19	12

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT1G72060	5.48	< 0.05	17	12	21	60	118	35	10	25	16
AT3G59310	6.78	< 0.05	42	45	83	263	360	263	61	48	34
AT3G05900	4.23	< 0.05	126	120	175	753	756	704	183	155	222
AT2G23100	9.47	< 0.05	9	3	20	86	51	39	5	7	5
AT1G63860	7.48	< 0.05	14	12	32	144	95	100	14	12	17
AT5G23210	5.57	< 0.05	134	140	291	1134	1125	717	219	200	162
AT5G18840	10.57	< 0.05	2	6	20	120	23	8	3	1	9
AT1G50040	9.54	< 0.05	5	18	65	198	209	45	6	33	10
AT5G01740	5.60	< 0.05	14	14	56	162	161	119	30	40	14
AT5G54610	16.80	< 0.05	15	5	11	41	37	110	5	2	3
AT5G44130	7.77	< 0.05	67	128	194	919	651	533	62	147	74
AT5G41080	4.10	< 0.05	24	21	28	239	184	17	26	44	46
AT4G31800	11.77	< 0.05	24	5	59	259	172	63	14	20	10
AT5G59670	7.78	< 0.05	9	5	13	31	31	124	11	6	4
AT4G37530	4.36	< 0.05	15	9	38	159	130	63	21	18	44
AT4G28085	8.30	< 0.05	6	4	8	42	40	29	1	7	5
AT1G11260	4.89	< 0.05	502	575	776	4343	4777	1664	468	1206	709
AT1G75750	7.99	< 0.05	379	199	772	3222	2862	1560	368	388	285
AT3G01290	13.60	< 0.05	145	27	143	666	825	362	37	41	72
AT4G18010	9.60	< 0.05	48	26	144	632	496	152	53	35	55
AT4G31000	5.84	< 0.05	10	14	62	192	185	137	31	28	33
AT4G26690	6.56	< 0.05	95	129	181	1030	732	707	117	142	142
AT4G39070	4.30	< 0.05	4	9	9	62	56	31	8	14	14
AT4G25110	6.90	< 0.05	9	6	8	46	39	75	5	8	9
AT5G25440	15.64	< 0.05	8	2	29	95	105	61	7	4	6
AT1G03457	7.19	< 0.05	12	10	19	110	108	94	14	13	18
AT5G57550	9.97	< 0.05	19	28	68	187	316	296	13	56	18

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT5G64100	5.24	< 0.05	3	3	16	110	69	7	5	1	30
AT3G47340	7.37	< 0.05	181	322	309	3203	2332	1403	143	561	281
AT1G62440	14.57	< 0.05	2	4	2	54	25	10	1	2	1
AT5G03615	6.62	< 0.05	3	2	8	44	54	18	2	13	3
AT1G21910	8.05	< 0.05	5	7	25	190	124	27	5	25	13
AT4G30270	9.91	< 0.05	228	132	351	2757	2389	1686	154	252	329
AT1G18020	5.94	< 0.05	7	26	41	302	277	115	42	57	30
AT3G45970	12.35	< 0.05	23	40	129	820	785	217	26	89	42
AT1G66160	10.24	< 0.05	5	2	8	79	62	21	8	5	3
AT2G35290	9.18	< 0.05	0	1	7	36	38	12	2	5	2
AT2G25770	18.97	< 0.05	2	0	6	43	43	4	0	2	2
AT3G30775	12.73	< 0.05	119	199	234	3240	2162	612	89	155	266
AT4G08950	7.95	< 0.05	55	103	189	1921	1379	542	69	339	94
AT4G21680	7.17	< 0.05	6	5	1	116	45	28	1	1	23
AT5G57560	8.85	< 0.05	50	68	253	1776	2261	431	69	375	92
AT4G16563	34.28	< 0.05	9	6	51	484	206	163	5	11	8
AT4G14130	10.42	< 0.05	17	31	32	645	314	207	22	35	59
AT1G19380	15.22	< 0.05	10	2	20	190	214	64	9	9	14
AT4G23070	16.02	< 0.05	0	1	0	11	19	2	0	1	0
AT4G30280	21.75	< 0.05	5	1	12	135	112	32	0	6	6
AT1G10550	30.82	< 0.05	3	0	10	142	83	51	3	3	2
AT4G25810	52.45	< 0.05	7	5	17	363	184	65	3	2	6
AT5G24030	31.27	< 0.05	13	13	46	780	502	176	13	15	19
AT1G35140	32.41	< 0.05	9	2	57	949	914	151	4	52	8
AT4G26260	14.13	< 0.05	1	1	0	111	49	0	1	0	10
AT2G19800	35.29	< 0.05	18	30	26	1586	1279	185	12	18	62
AT2G25510	5.16	< 0.05	1262	886	814	2262	1607	4535	891	562	265

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT4G02380	6.06	< 0.05	392	237	507	1291	1281	808	150	195	252
AT1G24147	3.04	< 0.05	36	15	42	48	73	98	37	29	15
AT3G54810	3.33	< 0.05	38	108	229	411	220	189	48	156	60
AT1G26210	3.07	< 0.05	35	29	47	106	87	70	55	22	21
AT1G57680	3.05	< 0.05	204	259	394	728	645	645	205	286	208
AT4G38550	4.35	< 0.05	178	160	231	503	356	488	103	118	127
AT1G27020	3.79	< 0.05	73	61	122	310	222	73	53	31	88
AT4G23270	3.02	< 0.05	62	51	118	198	159	200	98	41	61
AT1G61667	3.70	< 0.05	25	22	50	73	102	70	26	25	22
AT3G19680	3.66	< 0.05	53	249	440	831	719	262	48	416	48
AT5G62865	4.62	< 0.05	12	23	30	75	60	36	7	20	11
AT3G57450	3.64	< 0.05	63	51	93	166	262	116	37	84	37
AT5G07440	3.16	< 0.05	248	270	372	1017	955	421	233	269	325
AT2G30600	3.80	< 0.05	201	255	549	1069	969	542	233	264	246
AT4G37450	3.84	< 0.05	44	62	25	216	76	92	32	46	24
AT2G01190	3.23	< 0.05	71	85	133	334	196	284	83	94	88
AT1G23030	3.10	< 0.05	49	76	185	355	312	179	95	119	78
AT4G08850	3.98	< 0.05	92	62	166	332	267	303	76	83	79
AT5G14120	3.21	< 0.05	499	640	771	2271	1803	1229	558	639	597
AT4G09030	3.29	< 0.05	19	20	20	82	42	49	9	22	23
AT1G21270	4.34	< 0.05	121	120	224	413	471	445	139	105	87
AT3G50480	4.40	< 0.05	67	42	103	212	261	138	48	38	65
AT2G17230	4.64	< 0.05	67	176	459	839	611	487	86	276	70
AT3G60320	3.47	< 0.05	167	168	366	766	587	644	232	180	202
AT1G31540	4.13	< 0.05	58	45	123	180	217	256	77	26	68
AT3G23750	3.19	< 0.05	86	87	155	425	288	260	103	110	110
AT5G08760	4.53	< 0.05	34	22	67	105	147	103	45	27	21

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT3G15356	8.12	< 0.05	30	17	23	69	78	68	7	10	10
AT4G08470	3.20	< 0.05	66	52	145	203	269	289	118	69	75
AT3G11280	3.17	< 0.05	62	32	64	165	172	140	75	47	43
AT2G41330	3.44	< 0.05	19	20	30	76	68	53	17	28	13
AT4G22305	4.36	< 0.05	12	10	25	43	49	50	14	10	10
AT2G41110	3.14	< 0.05	45	46	69	193	179	121	51	58	55
AT4G32790	3.56	< 0.05	22	17	73	108	136	83	34	31	34
AT2G30990	4.16	< 0.05	29	40	54	131	135	119	44	24	31
AT5G51550	3.82	< 0.05	313	510	636	1878	1516	1061	291	624	309
AT4G38400	3.58	< 0.05	19	30	42	135	83	59	18	36	28
AT1G14520	3.31	< 0.05	14	13	69	99	114	75	28	30	33
AT5G66200	3.77	< 0.05	107	126	206	565	389	403	107	148	124
AT3G28180	3.81	< 0.05	90	129	205	552	405	357	102	155	107
AT3G28200	3.63	< 0.05	47	46	88	221	214	122	49	68	46
AT3G27960	3.24	< 0.05	37	54	70	201	181	124	46	67	51
AT4G37520	3.01	< 0.05	111	105	211	731	426	161	105	105	267
AT5G03120	4.09	< 0.05	50	69	111	197	322	201	67	81	41
AT3G58620	3.31	< 0.05	29	42	83	170	184	122	54	52	48
AT4G04570	4.05	< 0.05	51	56	93	215	215	207	57	59	49
ATMG01380	8.10	< 0.05	2	5	0	3	2	4	0	1	0
AT4G33050	3.99	< 0.05	122	31	88	132	128	113	40	23	37
AT1G75945	9.20	< 0.05	15	1	1	0	2	30	1	2	0
AT1G19020	5.71	< 0.05	20	3	19	21	39	8	1	4	7
AT1G15125	3.73	< 0.05	34	13	36	46	51	43	7	12	19
AT3G22235	3.32	< 0.05	1651	1823	987	2162	2202	4415	1350	1113	337
AT5G42530	3.42	< 0.05	1397	1239	1111	2076	1546	4260	1127	940	358
AT1G04107	3.24	< 0.05	27	23	32	70	56	37	16	31	6

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT1G55450	3.62	< 0.05	131	45	115	176	218	189	68	54	52
AT3G22231	3.29	< 0.05	687	888	496	1250	964	2234	726	496	222
AT4G36670	3.52	< 0.05	76	64	83	230	150	73	37	44	55
AT1G24530	4.21	< 0.05	8	23	29	54	48	25	12	5	14
AT2G07774	3.46	< 0.05	11	7	13	19	27	16	4	9	8
AT1G31173	5.63	< 0.05	2	2	10	11	9	8	2	2	1
AT4G14365	9.81	< 0.05	55	2	50	72	92	65	7	11	5
AT2G30250	4.77	< 0.05	76	18	52	131	118	73	29	19	24
AT3G22060	3.26	< 0.05	35	25	18	73	56	46	24	24	11
AT4G18205	4.03	< 0.05	58	24	61	109	113	100	21	33	29
AT1G74440	5.72	< 0.05	16	34	34	51	45	70	16	17	8
AT1G13210	3.36	< 0.05	68	34	77	155	135	145	50	32	53
AT2G32160	3.07	< 0.05	27	25	26	49	62	100	36	17	15
AT5G10695	3.20	< 0.05	14	8	13	26	50	9	6	15	8
AT1G17430	4.09	< 0.05	16	22	21	50	57	46	20	11	8
AT1G61260	3.40	< 0.05	15	17	28	34	63	51	7	25	13
AT5G11160	4.38	< 0.05	13	16	24	43	61	39	10	15	8
AT5G44568	7.04	< 0.05	6	4	10	18	18	16	4	5	3
AT3G46280	3.49	< 0.05	12	13	36	111	51	3	8	4	36
AT2G25735	5.95	< 0.05	17	7	9	33	45	15	7	6	3
AT3G25882	5.60	< 0.05	5	5	7	12	13	23	0	8	0
AT2G22470	5.49	< 0.05	4	2	18	28	23	14	4	1	7
AT5G58670	6.26	< 0.05	18	23	53	87	111	58	15	5	22
AT4G21850	4.50	< 0.05	19	2	9	36	26	27	6	8	6
AT5G46330	5.32	< 0.05	36	30	74	139	107	158	28	25	24
AT2G39210	4.32	< 0.05	24	13	41	80	62	97	20	10	27
AT5G51670	3.76	< 0.05	15	13	19	63	45	49	16	16	11

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT4G02330	7.19	< 0.05	9	10	14	52	45	15	7	6	3
AT2G46430	8.59	< 0.05	28	13	20	49	62	116	15	2	8
AT5G44820	7.17	< 0.05	7	5	12	20	30	34	6	3	3
AT3G26200	4.14	< 0.05	11	14	8	30	32	74	11	7	15
AT4G11000	15.07	< 0.05	6	1	7	23	28	18	0	3	0
AT3G45060	6.95	< 0.05	3	3	10	69	8	13	7	4	3
AT4G23220	16.76	< 0.05	4	2	5	22	29	17	0	1	1
AT1G65790	11.75	< 0.05	7	1	9	28	42	51	4	3	1

APPENDIX D. TRANSCRIPTS UPREGULATED IN ZNONP TREATED PLANTS COMPARED TO UNTREATED CONTROL PLANTS AND ZN^{+2} ION TREATED CONTROL PLANTS

Feature ID	Ctrlvs10_FC	Ctrlvs10_FDR	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT5G56550	3.14	< 0.05	-5.10	< 0.05	102	42	52	39	57	32	311	197	220
AT1G69490	4.43	< 0.05	-4.95	< 0.05	15	7	7	10	10	9	65	33	64

APPENDIX E. TRANSCRIPTS UPREGULATED IN RESPONSE TO THE ZNONP TREATED PLANTS COMPARED TO THE ZN^{+2} ION TREATED CONTROL PLANTS

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT1G22770	-11.4768	< 0.05	114	88	117	3	4	11	107	81	83
AT1G74930	-10.9811	< 0.05	54	16	14	1	3	5	48	38	34
AT5G15960	-5.73721	< 0.05	24	37	14	3	5	2	33	21	14
AT1G29910	-3.84089	< 0.05	16134	20683	16380	2269	2552	2960	12066	11464	8251
AT2G05100	-3.67685	< 0.05	825	721	609	91	122	107	478	341	463
AT1G71030	-11.8718	< 0.05	109	57	33	16	10	6	179	115	135
AT5G52310	-3.31429	< 0.05	215	151	175	24	20	43	152	85	82
AT2G08665	-4.46742	< 0.05	12950	15848	9506	2092	1227	3279	7854	15237	6684
AT5G24150	-11.3459	< 0.05	191	188	200	31	28	34	508	285	367
AT5G54585	-9.81473	< 0.05	20	14	11	0	6	0	33	19	32
AT2G34420	-3.27155	< 0.05	12827	15614	9217	2009	1230	3294	7780	7579	6887
AT5G54270	-3.37499	< 0.05	5448	4936	5488	744	930	972	3815	2769	3043
AT1G73600	-3.77244	< 0.05	586	412	589	82	93	100	512	293	336
AT3G59400	-3.50446	< 0.05	43	94	48	5	13	20	41	68	35
AT1G07050	-4.34224	< 0.05	207	174	242	34	35	63	242	202	168
AT5G15970	-3.96232	< 0.05	2427	2865	3763	472	781	633	3449	3030	1581
AT1G56600	-8.40565	< 0.05	58	30	58	4	9	17	116	84	86
AT5G02810	-3.8252	< 0.05	118	87	237	32	22	34	150	81	146
AT1G18710	-3.83784	< 0.05	46	74	94	7	16	25	94	79	33
AT3G26290	-4.00071	< 0.05	72	44	78	16	8	19	72	39	77
AT4G16880	-4.8123	< 0.05	55	41	51	7	9	17	67	48	60
AT4G19170	-6.44732	< 0.05	219	135	156	32	25	62	307	234	287
AT2G40080	-4.10989	< 0.05	199	174	223	56	31	56	204	224	192
AT1G13930	-3.71433	< 0.05	3595	3606	3684	826	954	887	3895	3922	2763
AT3G14210	-3.02886	< 0.05	5387	5592	7238	1595	1280	1664	5848	4170	4681
AT1G11210	-4.32525	< 0.05	57	40	54	18	7	13	71	39	70
AT5G03230	-5.62372	< 0.05	103	40	51	16	11	25	118	64	136
AT5G18010	-4.29836	< 0.05	14	19	21	3	2	9	14	32	18
AT2G41870	-4.7658	< 0.05	64	49	49	16	7	23	89	70	71

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT5G18030	-4.52846	< 0.05	24	16	33	3	8	9	26	48	25
AT1G60590	-6.20739	< 0.05	98	95	67	18	18	40	233	135	156
AT1G06040	-3.98311	< 0.05	215	175	134	44	40	74	244	212	217
AT5G64770	-3.30618	< 0.05	475	315	460	75	181	122	499	559	281
AT5G24155	-4.04346	< 0.05	103	108	151	34	37	37	231	146	103
AT3G62070	-3.74501	< 0.05	38	35	27	5	17	9	43	52	35
AT3G62550	-3.26057	< 0.05	1059	951	1411	311	459	342	1446	1446	1082
AT2G32290	-4.19416	< 0.05	77	39	76	13	22	31	150	50	104
AT3G07350	-5.18449	< 0.05	141	91	69	38	29	40	190	152	256
AT3G21670	-3.83075	< 0.05	186	139	137	59	35	74	236	163	292
AT1G78995	-3.40598	< 0.05	121	107	121	34	39	50	196	126	140
AT1G68050	-3.0498	< 0.05	123	90	111	21	34	63	157	104	128
AT3G17790	-4.32404	< 0.05	106	83	79	26	28	44	118	131	206
AT4G33666	-3.21521	< 0.05	118	85	70	45	27	40	126	118	139
AT2G40610	-3.02341	< 0.05	216	216	158	44	57	143	312	237	238
AT4G11360	-3.73813	< 0.05	95	75	49	23	31	43	146	109	137
AT2G46680	-4.25422	< 0.05	111	63	101	53	34	32	191	128	233
AT3G63210	-3.24102	< 0.05	157	124	117	44	51	98	260	179	236
AT3G08165	-35.5925	< 0.05	0	1	1	0	0	0	0	1	2
AT2G21320	-8.84739	< 0.05	11	9	5	0	1	3	16	19	12
AT3G47500	-6.05719	< 0.05	36	18	29	4	5	6	58	15	41
AT1G13650	-5.44836	< 0.05	46	22	44	7	8	10	72	44	43
AT3G63088	-15.6339	< 0.05	3	2	5	0	2	0	16	14	12
AT3G12580	-5.99237	< 0.05	68	37	33	16	8	13	71	51	126
AT5G03285	-4.89814	< 0.05	0	7	8	2	0	2	7	11	3
AT1G18330	-6.40682	< 0.05	20	13	16	4	6	4	36	31	42
AT5G18050	-3.66158	< 0.05	18	11	20	1	5	10	19	28	17
AT4G39366	-7.10735	< 0.05	1	1	1	0	0	1	1	1	6
AT5G10930	-4.66925	< 0.05	42	29	27	6	8	20	76	33	71

Feature ID	10vs19.7FC	10vs19.7FDR	Ctrl-R3	Ctrl-R2	Ctrl-R1	19.7ug_R3	19.7ug_R2	19.7ug_R1	10ug_R1	10ug_R2	10ug_R3
AT3G05030	-4.29166	< 0.05	58	46	75	25	17	27	122	67	139
AT5G59350	-3.27091	< 0.05	31	37	45	13	11	22	65	49	51
AT3G18773	-4.62363	< 0.05	36	19	10	13	4	11	39	36	66
AT3G27250	-4.43403	< 0.05	26	25	25	2	11	19	70	44	47
AT4G22200	-3.56432	< 0.05	91	87	109	27	33	63	209	108	174
AT1G01250	-4.03519	< 0.05	12	11	15	2	8	6	31	19	24
AT1G46554	-3.01036	< 0.05	25	24	33	15	8	13	48	34	36
AT3G28270	-4.88087	< 0.05	70	78	63	21	23	53	176	159	175
AT5G47610	-4.17653	< 0.05	37	19	15	11	10	12	43	58	48
AT5G20150	-3.28839	< 0.05	95	55	52	20	49	27	103	99	137
AT3G24500	-3.77768	< 0.05	12	22	33	9	13	11	48	42	50
AT5G59220	-3.7993	< 0.05	39	54	70	25	29	29	140	100	104
AT4G27410	-3.50665	< 0.05	48	30	16	23	20	7	70	41	81
AT1G80440	-3.3553	< 0.05	598	373	232	306	262	115	723	857	887
AT5G60680	-3.09665	< 0.05	185	156	175	99	97	110	426	234	371
AT1G76590	-3.20557	< 0.05	65	34	40	29	29	29	101	93	103
AT5G50450	-3.0851	< 0.05	72	47	59	46	36	28	153	91	126
AT3G29575	-3.05182	< 0.05	54	43	42	28	30	28	106	74	108
AT1G22640	-3.16029	< 0.05	45	30	31	21	23	37	96	83	98
AT5G15500	-3.31663	< 0.05	36	20	30	14	36	27	110	66	108

APPENDIX F. BIOLOGICAL PROCESS INVOLVED IN RESPONSE TO ZNONP TREATED PLANTS COMPARED TO UNTREATED CONTROL PLANTS AND ZN^{+2} ION CONTROL PLANTS

GO.ID	Biological Process	Genes	topGo Fisher
GO:0060919	auxin influx	1	< 0.05
GO:0010315	auxin efflux	1	< 0.05
GO:0010540	basipetal auxin transport	1	< 0.05
GO:0006952	defense response	5	< 0.05
GO:0006979	response to oxidative stress	2	< 0.05

APPENDIX G. BIOLOGICAL PROCESS INVOLVED IN RESPONSE TO THE ZNONP TREATED PLANTS COMPARED TO THE UNTREATED CONTROL PLANTS

GO.ID	Biological Process	Genes	topGo Fisher
GO:0009626	plant-type hypersensitive response	2	< 0.05
GO:0050832	defense response to fungus	2	< 0.05
GO:0098542	defense response to another organism	4	< 0.05
GO:0070417	cellular response to cold	1	< 0.05
GO:0009682	induced systemic resistance	1	< 0.05

APPENDIX H. BIOLOGICAL PROCESS INVOLVED IN THE ZNONP TREATED PLANTS COMPARED TO THE ZN $^{+2}$ ION TREATED CONTROL PLANTS

GO.ID	Biological Process	Genes	topGo Fisher
GO:0010411	xyloglucan metabolic process	10	< 0.05
GO:0042546	cell wall biogenesis	10	< 0.05
GO:0009751	response to salicylic acid	13	< 0.05
GO:0006979	response to oxidative stress	15	< 0.05
GO:0009741	response to brassinosteroid	9	< 0.05
GO:0010200	response to chitin	9	< 0.05
GO:0071555	cell wall organization	18	< 0.05
GO:0006468	protein phosphorylation	22	< 0.05

APPENDIX I. BIOLOGICAL PROCESS INVOLVED IN RESPONSE TO THE ZNONP TREATED PLANTS COMPARED TO THE ZN $^{+2}$ ION TREATED CONTROL PLANTS

GO.ID	Biological Process	Genes	topgoFisher
GO:0009768	photosynthesis, light harvesting in phot...	6	< 0.05
GO:0009737	response to abscisic acid	14	< 0.05
GO:0018298	protein-chromophore linkage	5	< 0.05
GO:0009644	response to high light intensity	5	< 0.05
GO:0009637	response to blue light	5	< 0.05
GO:0010218	response to far red light	4	< 0.05
GO:0010114	response to red light	4	< 0.05
GO:0007623	circadian rhythm	6	< 0.05