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By

Mohd. Zahid Rizvi and M.M. Abid Ali Khan

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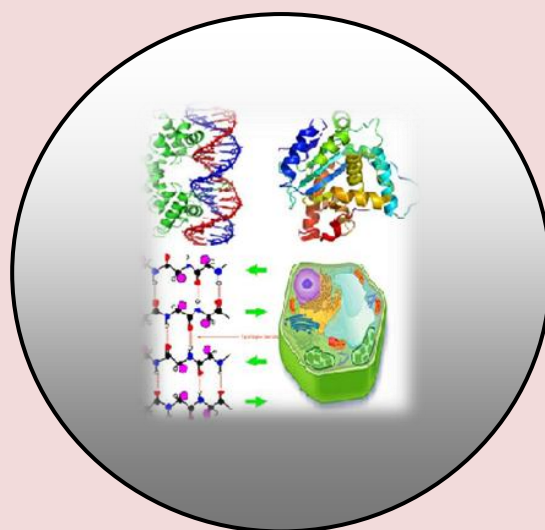
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Dr. Mohd. Zahid Rizvi

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## **Nanomaterials: Metabolism, Regulation and Functions in Crop Abiotic Stress Tolerance**

**Mohd. Zahid Rizvi and M.M. Abid Ali Khan**

Department of Botany, Shia Post Graduate College, Sitapur Road, Lucknow-226020,  
Uttar Pradesh, India

### **ABSTRACT**

*Abiotic stresses like drought, salt, chilling, heavy metal stress have serious impact on development and productivity of crops. There is an urgent requirement to mitigate negative effects of abiotic stress on crops for enhancing crop productivity to fulfil global food requirement. The plants cope with abiotic stress through alterations in their structure and physicochemical processes. Abiotic stress resistant varieties are developed through modern methods like genetic engineering. Nowadays, nanotechnology is helpful in counteracting effects of abiotic stress on plants. Nanomaterial synthesis can be achieved through different methods. The green manufacturing of nanomaterials involving plants through biological method seems promising. Nanomaterials, harbour properties not found in larger particles. These nanomaterial properties can play important role in tackling stress scenerios in crops thus improving their growth and productivity prospects under abiotic stress. But effects of this technology on crops are to be properly assessed and future work is required. Nanoparticles, being small sized, may be toxic to plants. Due to this globally risk assessment and regulatory methods are being developed but worldwide different approaches are employed to regulate use of nanotechnology in agriculture and nanobased agricultural products. A consensus should be reached globally for policies regarding toxicity, biomonitoring, risk assessment and regulation of nanomaterials, especially in agriculture and agricultural products. Present review paper takes into account nanomaterial's role for abiotic stress resistance in crops. Aspects like synthesis, metabolism, toxicity, regulation of nanomaterials are also covered.*

**Keywords:** Abiotic stress, nanomaterials, plants, regulation, synthesis and toxicity.

### **INTRODUCTION**

A mismatch is being created between food demand created by increasing global population and supply through agricultural production.

Cultivated land is shrinking mainly due to the use of agricultural land for urbanization, industrial and other anthropogenic activities which have significant adverse effect on crop productivity (Das and Das, 2019). Abiotic stress has major effect on decrease in crop productivity up to 70% (Acquaah, 2007). Developing countries not having much needed resources to tackle effect of abiotic stress on crops, bear the brunt of increasing hunger problems. Different kinds of abiotic stress encountered include drought, salt stress, heavy metals stress, waterlogging, low and high temperature stress, ozone and ultraviolet radiation. Biotic stresses in plants chiefly includes bacteria, virus and fungi (Abiri et al., 2017; Lu et al., 2017). Due to climatic alterations and impact of abiotic stresses on crop yield, effective strategies should be developed for abiotic stress resistance in plants (Wani et al., 2016). Conventional breeding strategies are not much effective in enhancing stress resistance of crops. Therefore, development of efficient strategies besides traditional methods like breeding, are urgently required (Das and Das, 2019).

Recently nanomaterials (NMs) are being employed in strategies for abiotic stresses resistance in plants (Reddy et al., 2016; de la Rosa et al., 2017). The nanotechnology, employed in creation of NMs, operates between 1 and 100 nanometers (nm); [Dubchak et al., 2010; Recommendation on the definition of a nanomaterial (2011/696/EU); Rai et al., 2018]. Nanoparticles (NPs) small size grants them easy access inside plants. NPs having miniscule size, enhanced surface area and catalytic action, can interact with plants more efficiently (Dubchak et al., 2010). Besides this, NPs are stable, have high adsorption, electrical and optical properties (Rai et al., 2018). Lynn W. Jelinski, USA, introduced term “nanobiotechnology” (Saxena et al., 2016). Possessing peculiar structural and functional features, NMs characters vary from ordinary compounds. Common types of NMs observed are carbon nanotubes, dendrimers, quantum dots, fullerenes, different metal and metal oxide NPs. NMs are manufactured artificially or generated through various natural processes (Das and Das, 2019). Many researches have been conducted on association between NMs and plant stresses (Kole et al., 2016; Zaytseva and Neumann, 2016). Abiotic stress exerts oxidative stress on plants (Servin and White, 2016). NMs may be helpful to plants undergoing stress in inducing defence system consisting mainly antioxidative enzymes (Patra et al., 2016). NPs increase the drought resistance through enhanced root water uptake in plants, neutralizing reactive oxygen species (ROS), stress signaling, and inducing relevant hormonal cascades. The small size and enhanced translocation capacity of NPs results in speedy delivery of nutrients throughout plants, thus enhancing their nutritional status during stress (Das and Das, 2019).

Nowadays NPs are synthesized artificially for impact on plant's growth by modifying their physiological and biochemical processes for many purposes including stress tolerance (Giraldo et al., 2014). The artificially manufactured NPs can take genetic materials like DNA into plant tissues and organs (Torney et al., 2007). NPs affect various crop yield, development and quality parameters (Burke et al., 2015; Jalil and Ansari, 2019), indicating towards possible advantages of nanotechnology in agriculture. Despite the enhanced NMs application in agriculture globally, the information of mechanism of NPs functions and interaction with plants is not at advanced stage (Khodakovskaya et al., 2011). In this review article, development and agricultural use of NMs for resistance and adaptation to abiotic stress is discussed. It also covers synthesis, uptake, movement, aggregation, interaction of NMs in plants and toxicity and regulatory aspects.

## **METABOLISM OF NANOMATERIALS**

### **Entry, Transport and Aggregation of Nanomaterials inside Plants**

Uptake, transport and accumulation of NMs is affected by many factors like plant type and size, kind, chemistry and stability of NPs. The movement and aggregation of NPs in plant depends on plant cell's structural and physiological aspects and how NMs behave during their uptake by the soil (Janmohammadi et al., 2016). Aggregation of non-toxic NPs depends upon exposure levels. There is a proportional relationship between transpiration rates and NPs movement (Hendrickson et al., 2017). Initially NPs uptake from soil occurs by roots, thereafter NPs move to aerial plant parts and stored within cell organelles (Nair et al., 2010). Many changes in NPs like crystal phase dissolution, biotransformation, bioaggregation takes place to facilitate their uptake and transfer to plant tissues. The NP's size is vital for uptake and entry into cellular and stomatal pores, movement into cells through plasmodesmata and into cellular organelles and it subsequently affects NPs toxicity to plants (Tripathi et al., 2017). Shape and surface area of NPs affect their agglomeration, reactivity on cell surface and inside plants (Wang et al., 2013). The NPs enter plants initially in roots and going through lateral root junctions reach xylem (Dietz and Herth, 2011); (Figure1). The NPs interact with plants through ROS generation, ion cell membrane transport and lipid peroxidation. Inside cells, NPs react with functional groups like sulfhydryl and carboxyl and subsequently change functionality of proteins. NPs movement in plants takes place through their attachment with membrane transporters or root exudates (Watanabe et al., 2008; Kurepa et al., 2010). Inside cytoplasmic organelles, NPs affect cellular physiology (Zhang and Monteiro, 2009). The transmission across generations of C70-NOM in rice plants was found after their treatment with fullerenes in first generation (Lin et al., 2009).

The nature of NPs affects their entry through cell wall and cell membrane or stimulating association with radical surface or radical exudates (Ling and Silberbush, 2002). Additionally structure and coating on NPs affects their movement in rhizosphere and interaction with plants. The charge on NPs and charge on cell wall, NPs size and hydrophobicity found on plant surface has impact on association of NPs with cell wall and their uptake and movement (Kaphle et al., 2018). Gold (Au) NPs with positive charge have quick uptake by roots. Au NPs with negative charge move from roots to shoots. Cell wall's pore size also impacts NPs uptake by the cell. It was reported that 40-50 nm size NPs move inside the cell (Mousavi et al., 2007). Additionally further small sized NPs (3 to 5 nm) can either directly move into root epidermal cells or they can move inside roots due to osmotic pressure, capillary forces. On the other hand, large NPs can not move inside semipermeable epidermal cells of root cell wall. Some NPs can move inside plants by inducing new pores in epidermal cell wall (Lin and Xing, 2008; Du et al., 2011). After passing through cell wall, NPs use extracellular spaces to arrive at central vascular tissue by apoplastic movement, permitting in xylem to move in single direction upside. But NPs have to symplastically pass through the casparian strip for their entry in central vascular cylinder. This is facilitated by attachment with endodermal cell membrane's carrier proteins, pore generation and movement. Intercellular NPs movement occurs through plasmodesmata as assimilated in cytoplasm (Figure 1); (Perez-de-Luque, 2017; Tripathi et al., 2017). NPs not moving further inside are stuck on casparian strip, while NPs arriving at the xylem move to shoots and through phloem again to roots (Wang et al., 2012; Perez-de-Luque, 2017).

Direct intake of NPs in seeds may take place by their movement inside coat through parenchymatic intercellular spaces, and diffusion in cotyledon (Tripathi et al., 2017). The entry points of NPs in leaves are stomata or cuticles. The cuticle inhibits the intake of NPs lesser than 5 nm. On the other hand NPs larger than 10 nm are taken up through stomata, and their cellular movement takes place by apoplastic and symplastic passages into plant vascular system (Ruttkey-Nedecky et al., 2017); (Figure 1). The movement of NPs (10-50 nm), preferentially takes place through the symplastic passage (neighbor cell's cytoplasm). While larger NPs in size range 50-200 nm have intercellular movement (apoplastic passage). Sugar flow passing through phloem sieve tubes helps in the movement of NPs which come inside plants. Phloem transport helps in bidirectional movement of NPs which aggregate in sink organs for sap e.g. roots, shoots, fruits, grains and foliage (Wang et al., 2013; Ruttkey-Nedecky et al., 2017). Numerous nutrients, nonessential metal complexes prefer nonselective apoplastic passage (Banijamali et al., 2019). The efficient NPs entry in leaves after foliar application depends on how they are applied, their size, concentration and climatic conditions (Wang et al., 2013). Morphology of leaves, their chemical composition, trichomes, leaf exudates and waxes help in NPs attachment on leaf surface (Larue et al., 2014). Climatic conditions also affect aggregation rate of NPs in roots. Addition of potassium chloride and ammonium thiosulfate to silver sulfide (Ag<sub>2</sub>S) NPs increased AgNPs accumulation in *Lactuca sativa* shoot and roots (Doolette et al., 2015).

Bioavailability and toxic properties of NPs depend upon their biotransformations in soil. NPs uptake also depend upon the plant species and characteristics of NPs itself. It was reported that Au NPs can accumulate in *Oryza sativa*. But they cannot aggregate inside *Cucurbita pepo* and *Raphanus raphanistrum* (Zhu et al., 2012). In *Cucurbita pepo*, silver (Ag) level in plant shoots was higher as compared to those plants which were treated with Ag powder exposed to 10-1000 mg L<sup>-1</sup> Ag NPs (Stampoulis et al., 2009). Titanium dioxide (TiO<sub>2</sub>) and silicon dioxide (SiO<sub>2</sub>) NPs were most stable NPs (Larue et al., 2011; Servin et al., 2012). Capacity for transformation by disparity has been observed in copper (II) oxide (CuO), cerium oxide (CeO<sub>2</sub>), lanthanum oxide (La<sub>2</sub>O<sub>3</sub>), and nickel oxide (NiO) NPs, which subsequently caused alterations in accumulated plant speciation (Castillo-Michel et al., 2017). In *Z. mays*, roots and shoots are the organs where highest storage of zinc (Zn) takes place when there is hydroponic application of zinc oxide (ZnO) NPs in different forms e.g. Zn-phosphate. The reason may be enhanced rhizospheric dissolution, plant absorption and Zn movement in ionic form during hydroponic exposure (Lv et al., 2015). Similar type of Zn aggregated speciation was reported in soil grown wheat (Dimkpa et al., 2012, 2013). During uptake from soil of ZnO and CeO<sub>2</sub> NPs into *Glycine max* while CeO<sub>2</sub> NPs moved as NPs, but biotransformation of Zn into Zn-citrus took place inside tissue (Hernandez-Viezcás et al., 2013). In *Z. mays* copper oxide (CuO) NPs moved from roots to shoots via xylem while they reverted via phloem (Wang et al., 2012). In *Triticum aestivum*, role of NP TiO<sub>2</sub> in translocation was studied. In *T. aestivum*, NPs size was very important in their movement and storage in different plant tissues like shoots and roots (Larue et al., 2012).

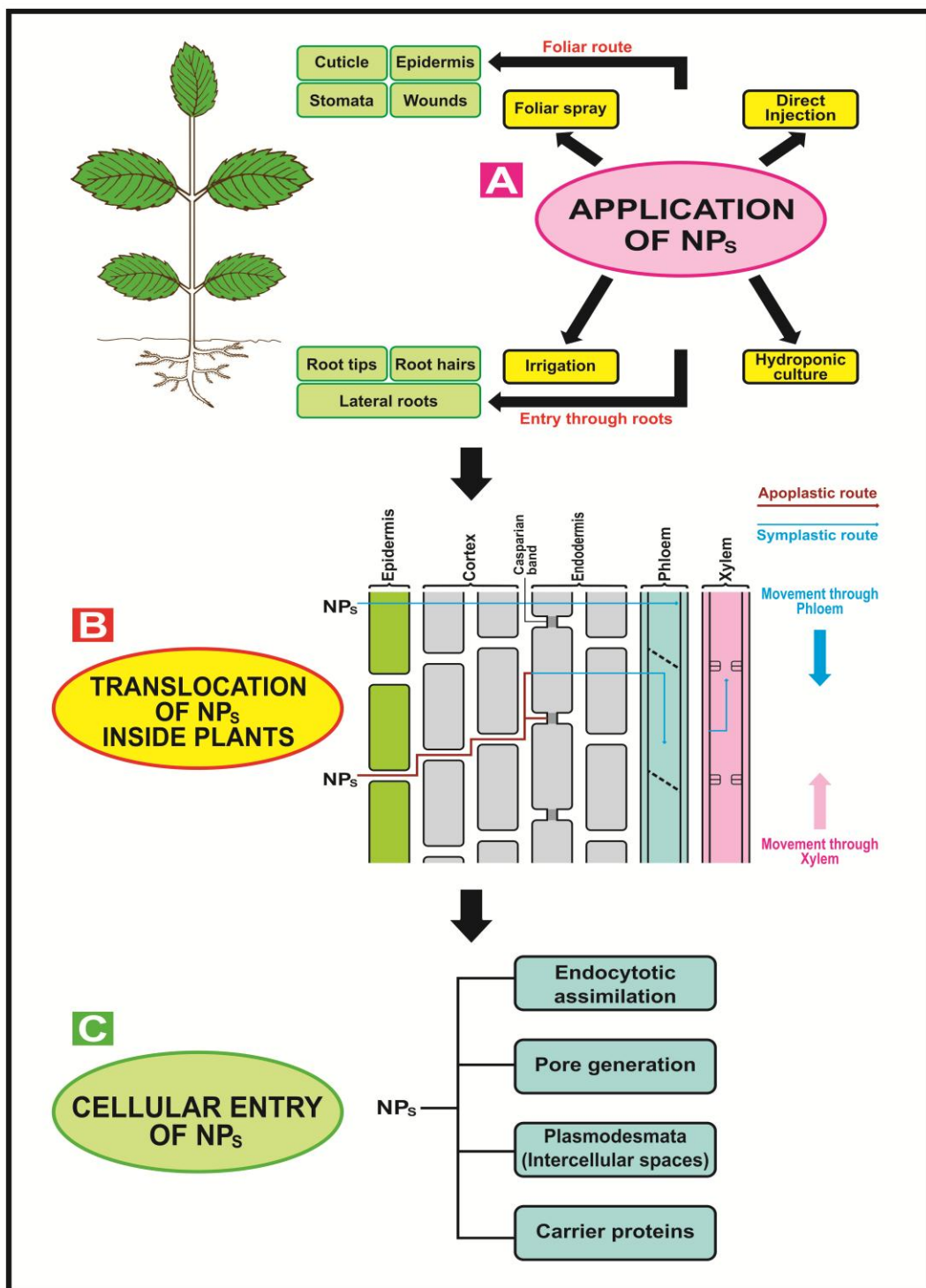


Figure 1. Uptake, inside movement and cellular entry of nanoparticles (NPs) in plants.

## Nanomaterials and Plants Interaction

Plants and NMs Interactions are influenced by plants species, growth medium, time period for which plants are exposed to NMs, exposure route, stress, NM physicochemical properties (Dietz and Herth, 2011; Ma et al., 2015). Additionally adsorption of NMs, assimilation, movement and bioaccumulation also impact their effect on plants (e.g., yield of crops, nutritional quality, NMs toxicity to plants and NMs movement to humans). The NMs can move across various trophic levels, internalized by edible plants and at higher levels can reach humans. Movement of NPs from algae and tobacco to the next trophic levels are observed (Navarro et al., 2008; Judy et al., 2011). NMs may be trapped on the waxy cuticle and move inside plants through pores such as stomatal pores. Water and nutrient elements in soil or hydroponic growth media help in NPs uptake in roots. External elements such as growth medium (Korenkova et al., 2017), root exudates (Rossi et al., 2018), pH (Du et al., 2017), cation exchange power (Xue et al., 2018) and mycorrhizal fungi (Noori et al., 2017) affect NPs intake. For entry through root and foliar routes, NM intake depends upon plant's type and transpiration rate (Zhao et al., 2017; Kranjc et al., 2018), size of NMs (Noori et al., 2017), surface functionalization (Deng et al., 2017); NM chemistry (Pagano et al., 2017; Pradas Del Real et al., 2017), stability (Ma et al., 2017a) and age (Gao et al., 2018).

Co-exposures of engineered NMs (ENMs) with pollutants and other kinds of ENMs impacts intake and movement of these substances by plants, thus influencing interactions of NMs with plants. ENMs when applied along with pollutants can amplify intake and adverse effects of pollutants in plants (Li et al., 2018). Intake of pollutants and toxicity can be reduced if concentration and/or access of pollutants to plants are decreased by adsorption and entrapment on surfaces of ENMs (Deng et al., 2017). Co-application of tetracycline with  $\text{TiO}_2$  in hydroponic medium resulted in inhibition of intake of tetracycline (Ma et al., 2017a). In another study,  $\text{SiO}_2$  NMs helped in overcoming negative effects of Nickel oxide (NiO) NMs on plant biomass and antioxidant capacity and reverted effects of NiO NMs on photosynthesis in barley plants (Soares et al., 2018). Rossi et al. (2018) observed that shoot cadmium (Cd) level was decreased in soybean hydroponic cultivation by co-application of polyvinylpyrrolidone (PVP)- $\text{CeO}_2$  NMs and  $\text{Cd}^{2+}$ . The reason for decreased shoot Cd level was attachment of biomolecules in root exudate and  $\text{Cd}^{2+}$ . Changes in Na levels in roots and foliage in rapeseed were observed following co-application of PVP- $\text{CeO}_2$  NMs and NaCl. These changes may be attributed to shortened root apoplastic barrier entities (Rossi et al., 2017). Therefore in plants, co-exposure of NMs with other chemicals may change the effects of both materials especially NMs.

### Biotransformation of NMs

Biotransformations of nanomaterials occur due to interactions between NMs and biota. How NMs act may be changed after NM biotransformations. Some important processes coming under NM biotransformations are dissolution, redox processes and chemical reactions with molecules occurring in contact with biological entities (Maurer-Jones et al., 2013). As far as ENM biotransformations are concerned, there are largely two categories. In first category there are ENMs who generally exhibit no changes under various environmental and biological scenarios, while in second category comes those ENMs which can undergo transformations. It was observed that dissolution of NMs are important for their biotransformation. Undissolved NMs are unlikely to undergo biotransformation (Cruz et al., 2017; Peng et al., 2017).

Hydroponic medium was found more suitable for NM uptake, accumulation, dissolution and biotransformation than soil. Therefore NMs have more chances to affect plants grown in hydroponic medium. Consistent with above mentioned observations, CeO<sub>2</sub> NM biotransformation was not reported after application of CeO<sub>2</sub> NMs to wheat (Rico et al., 2017) and tomato (Layet et al., 2017) roots in soil. In cucumber (Ma et al., 2017b) and wheat roots (Spielman-Sun et al., 2017) Ce(IV) to Ce(III) biotransformation was reported in hydroponic medium. In application of Ag NMs or silver sulfide (Ag<sub>2</sub>S) NMs to wheat root in hydroponic medium, biotransformation of both NMs was observed. It was revealed that dissolution of Ag<sub>2</sub>S NMs before uptake (may be because of root exudates), resulted in biotransformation of even highly stable Ag<sub>2</sub>S NMs (Pradas Del Real et al., 2017). In bean seed germination experiments with aqueous ZnO NMs, it was observed that biotransformed Zn amount was linked with the intensity of negative effects instead of total Zn found inside seedlings (Savassa et al., 2018). Therefore ease of dissolution of NMs has impact on effects of NMs on cultivated plants in soil. The dissolved ions were more harmful than the NMs. Treatment of leaves with NM-containing suspension gave better results instead of spraying (Borgatta et al., 2018).

### **TYPES OF NANOMATERIALS**

One criteria of classification of nanomaterials depends upon their dimensions: 0D category NMs are those whose entire dimensions belong to nanoscale, 1D category NMs have their only one dimension belonging to macroscale, examples of this category are nanofibers and nanowires, 2D and 3D covers NMs having their 2 and 3 dimensions in macroscale respectively; nanosheets and thin films comes under 2D category and materials in bulk belong to 3D category (Singh, 2016). Different forms in which NMs may be found are single, fused or agglomerated and various shapes are circular, tubular and irregular shape (Das and Das, 2019). Another criteria for classification of NMs is their chemical nature. Based upon this criteria, there are 4 types of NMs: carbon, metal, metal oxides and polymeric substances (Khan et al., 2019). Fullerenes, graphene and carbon nanotubes (CNTs) comes under carbon-based NMs (Ealias and Saravanakumar, 2017); Another type are inorganic metal-oxide compounds e.g. TiO<sub>2</sub>, ZnO and Iron oxide (FeO<sub>2</sub>) (Thomas et al., 2015); metallic NMs are based upon Au, Ag, Cu and Ni. Organic NMs consists of dendrimers, generated from organic NPs symmetrical to nucleus (Ealias and Saravanakumar, 2017). But in agriculture, usually carbon NMs, metal NPs and metal oxides NPs are employed.

#### **Carbon-based NMs**

Carbon-based nanomaterials (CNMs) have been extensively employed in agriculture (Zaytseva and Neumann, 2016). CNMs are stable, having enhanced chemical reactivity and they uniformly disperse in the medium (Verma et al., 2019). CNMs especially carbon nanotubes (CNTs) and fullerol are useful in stimulating drought resistance in many agricultural crops. Carbon-based NMs like CNTs, fullerenes and graphene can also be employed in various areas including precision agriculture (Zaytseva and Neumann, 2016).

#### **Metallic NPs**

Metallic NPs are employed in many areas-such as in medical diagnostic services, as antibacterials, having electrical and optical properties. Commonly encountered metallic NPs are made up of metals Au, Ag, Pt, Zn and Ni (Intermetallic alloys comes under metallic NPs); (Dolez, 2015). NPs made up of largely inert metals such as gold become more active after reduction in size at NP level, thus becoming useful in catalytic applications (Saleh, 2020).



### **Metal Oxide NPs**

Many metallic oxide compounds such as TiO<sub>2</sub>, ZnO, Tin (IV) oxide (SnO<sub>2</sub>), Ferric oxide (Fe<sub>2</sub>O<sub>3</sub>), Copper (II) oxide (CuO), Zirconium dioxide (ZrO<sub>2</sub>) and Molybdenum trioxide (MoO<sub>3</sub>) have been found to harbour photocatalytic properties (Sharma et al., 2018). Photocatalytic compounds on interaction with light of specific energy promote an electron and subsequently causing excited electron to generate hydroxyl radicals and other ROS, associated with photocatalytic degradation processes (Prasad et al., 2019). Alteration of metal oxide NPs surface by processes such as metal ion doping or non-metal insertion increases photocatalytic response (Bishoge et al., 2018).

### **NANOMATERIALS SYNTHESIS**

Physical, chemical or biological methods are employed for synthesis of NMs e.g. nanoparticles (Singh et al., 2016). Metals (Ag, Au etc) or metal oxides (TiO<sub>2</sub>, SiO<sub>2</sub>, ZnO) are usually taken for nanoparticles manufacturing but recently green synthesis of NPs through biological methods is becoming popular where mostly plants or plant extracts are employed. Green manufacturing of NPs is without threat to environment, cost-effective, without chemical impurities, and largely free from side-effects for biological uses (Gopinath et al., 2014).

### **Biosynthesis of NMs**

There are two methods for NMs synthesis. First one involves 'bottom-up' approach where starting from minute atoms and molecules, nanoscale materials are generated. Another approach is 'top-down' approach where starting from macro-level materials, going down the size-scale, small nano-scale materials are generated (Das and Das, 2019). Biological methods for NPs synthesis follow "bottom-up" techniques involving NPs generation from small-scale materials by reduction and oxidation and NPs manufactured through these processes are with fewer defects. Enzymes, sugars, proteins, secondary metabolites e.g. phenolics, terpenoids, flavonoids and latex, alcohols, amines and cofactors found in plants act as reducing and stabilizing agents during generation of NPs (Sharma et al., 2009; Siddiqui et al., 2014). Dihydroxy(oxo)titanium [TiO(OH)<sub>2</sub>] solution in conjunction with *Eclipta prostrata* leaf extract was employed for TiO<sub>2</sub> NPs synthesis (Rajakumar et al., 2012). Synthesis of CeO<sub>2</sub> NPs was achieved employing organic agarose polymer (Kargara et al., 2015). *Malva sylvestris* reduced Cu ions and this process resulted in generation of CuO NPs which have antibacterial properties (Awwad et al., 2015). Fe NPs synthesis employing green tea and eucalyptus leaf extract was reported by Wang et al. (2014). The extracellular synthesis of Ag, Au and Au-Ag NPs in water employing extract of mushroom *Volvariella volvacea* was achieved (Philip, 2009).

### **APPLICATIONS OF NANOMATERIALS IN ABIOTIC STRESS RESISTANCE OF CROPS**

Development and yield of different crops is adversely impacted by drought, salinity, temperature fluctuations, waterlogging and toxicity by mineral elements or their deficiencies (Boyer, 1982). Drought and salinity may reduce crop yield upto 50% (Kaushal and Wani, 2016). Water shortage and increasing high temperatures are causing environmental changes globally which decrease fertile agricultural area worldwide (Xue et al., 2016). Drought and salt stresses affect alterations in cellular structure and cell organelle structure e.g. chloroplast (Xu et al., 2009; Hu et al., 2018), these stresses also affect plant water relations and metabolism, cause alterations in nutritional balance.

**Table 1. Role of nanomaterials in mitigating various abiotic stresses in plants.**

Nanomaterial	Plant Species	Type of Abiotic Stress	Observed Response(s)	References
Nano-SiO <sub>2</sub>	Hawthorns ( <i>Crataegus</i> sp.)	drought	Effect of Nano-Si (silicon) during drought, on photosynthetic rate and stomatal conductance of Hawthorns ( <i>Crataegus</i> sp.) plants was observed. Nano-Si enhanced biomass, xylem water potential and malondialdehyde (MDA) levels. Therefore Nano-Si had positive effect in maintenance of important physiological and biochemical activities in hawthorn seedlings and increased plant's resistance for drought stress.	Ashkavand et al., 2015
Nano-Si	Rice ( <i>Oryza sativa</i> L)	Heavy metal (Pb) toxicity	Nano-Si exhibited better results as compared to common Si regarding alleviation of the toxicity induced by lead (Pb) on growth of rice and it reduced roots to shoots Pb movement and its storage in grains, especially in soils harbouring increased Pb levels and high-Pb accumulating-cultivars.	Liu et al., 2015
Nano-Si	Faba Bean ( <i>Vicia faba</i> L.)	Salinity	Nano-silicon treatments has been observed to decrease negative effects of salinity on <i>V. faba</i> by increasing activity of antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) in leaves, but reduced activity of superoxide dismutase (SOD) in plants without stress.	Qados, 2015

Nano-Si	( <i>Lens culinaris</i> Medik.)	Salinity	Applying Si-NPs on lentil ( <i>Lens culinaris</i> Medik.) genotypes exposed to salinity stress caused increase in seed germination and seedling growth.	Sabaghnia and Janmohammad, 2015
Nano-Si	Tomato ( <i>Solanum lycopersicum</i> L)	Salinity	Tomato seeds germination, root length and fresh weight of tomato seedlings experiencing salt stress increased. Salt stress genes <i>AREB</i> , <i>TAS14</i> , <i>NCED3</i> and <i>CRK1</i> were upregulated.	Almutairi, 2016b
AgNPs	Tomato ( <i>Solanum lycopersicum</i> )	Salinity	Seed germination, root length and seedling weight in tomato were enhanced after AgNPs treatment of plants undergoing NaCl induced salinity stress. <i>AREB</i> , <i>MAPK2</i> , <i>P5CS</i> and <i>CRK1</i> genes, have enhanced expression and <i>TAS14</i> , <i>DDF2</i> , <i>ZFHD1</i> genes, have decreased expression.	Almutairi, 2016a
AgNPs and Polyethylene glycol (PEG)	lentil ( <i>Lens culinaris</i> Medic)	Drought	Application of AgNPs along with PEG substantially affected germination, root length, dry and fresh weight in seeds of lentil. AgNPs application was helpful in increasing lentil germination under drought stress.	Hojjat, 2016
Nano-ZnO	Sunflower ( <i>Helianthus annuus</i> )	Water stress	Nano ZnO treatment substantially enhanced seed yield chiefly by increasing seed number per head and also increased water use efficiency.	Seghatoleslami and Forutani, 2015

ZnO NPs	Sorghum ( <i>Sorghum bicolor</i> )	Drought	Applying ZnO NP to soil enhanced development and yield, helped in fortifying grains with important essential nutrients like Zn, and improved nitrogen uptake capacity in sorghum exposed to drought.	Dimkpa et al., 2019
Nano-ZnO	Maize ( <i>Zea mays</i> L)	Drought	Drought caused changes in subcellular entities and storage of MDA and osmotically active materials. Application of nano-ZnO at 100 mg L <sup>-1</sup> concentration enhanced melatonin synthesis and antioxidant enzyme machinery due to which there was alleviation of injury to subcellular structures caused by drought in maize. Possibly alterations in endogenous melatonin synthesis were linked with nano-ZnO stimulated drought resistance in maize.	Sun et al., 2020
Copper (Cu) and zinc (Zn) NPs	Wheat ( <i>Triticum</i> sp.)	Drought	Cu and Zn NPs helped in overcoming drought impact on wheat by inducing antioxidant enzyme machinery and relative water level, reducing thiobarbituric acid reactive substance (TBARS) aggregation and by maintaining photosynthetic pigment level in leaves.	Taran et al., 2017
Nano-TiO <sub>2</sub>	Wheat ( <i>Triticum aestivum</i> L.)	Water deficit stress	Application of TiO <sub>2</sub> NPs through foliar route at 0.02% enhanced various agricultural parameters under water shortage conditions.	Jaberzadeh et al., 2013

Nano-TiO <sub>2</sub>	Chick pea ( <i>Cicer arietinum</i> L.)	Cold	Treatment of Nano-TiO <sub>2</sub> to chick pea ( <i>Cicer arietinum</i> L.) cold-sensitive and cold-tolerant genotypes experiencing cold stress exhibited that Nano-TiO <sub>2</sub> treatment did not stimulate oxidative damage and helped in coping with membrane damage during cold stress exposure.	Mohammadi et al., 2013
Nano-TiO <sub>2</sub>	Borage ( <i>Borago officinalis</i> L.)	Water shortage stress	Foliar application of methanol (45% v/v concentration) and nano TiO <sub>2</sub> (0.05%) in borage ( <i>Borago officinalis</i> L.) under less irrigation conditions resulted in maximum levels of chlorophyll (Chl) a, b and total chlorophyll, net photosynthetic rate, rubisco carboxylase activity, anthocyanin and nitrate reductase (NR).	Akbari et al., 2014
Nano-TiO <sub>2</sub>	Soybean ( <i>Glycine max</i> )	Heavy metal Cadmium (Cd)	Treatment of soybean plants with nano-TiO <sub>2</sub> reduced toxicity of Cd and Cd stress through enhancement in photosynthetic rate and growth characteristics and nano-TiO <sub>2</sub> in soil also increased Cd uptake by the plants.	Singh and Lee, 2016
Nano-TiO <sub>2</sub>	Moldavian balm ( <i>Dracocephalum moldavica</i> L.)	Salinity	In Moldavian balm, as compared to exposure to salinity stress scenarios without TiO <sub>2</sub> NPs treatment; TiO <sub>2</sub> NPs application under salt stress improved all agronomic characters and enhanced antioxidant enzyme activity. Nano-TiO <sub>2</sub> also largely reduced H <sub>2</sub> O <sub>2</sub> content. Highest essential oil level was observed in 100 mg L <sup>-1</sup> TiO <sub>2</sub> NP-treated plants under control conditions.	Gohari et al., 2020

Nano-TiO <sub>2</sub> and nano-SiO <sub>2</sub>	Cotton ( <i>Gossypium barbadense</i> L.)	Drought	Nano-TiO <sub>2</sub> or nano-SiO <sub>2</sub> treatment through foliar route to cotton plants experiencing drought stress resulted in enhancement of pigments content, total soluble sugars, phenolics and soluble proteins, proline levels, reducing power, antioxidant capability and increase in yield.	Shallan et al., 2016
γ-Fe <sub>2</sub> O <sub>3</sub> (maghemite) nanoparticles	<i>Brassica napus</i>	Drought	Treatment of Yttrium doping-stabilized γ-Fe <sub>2</sub> O <sub>3</sub> NPs to <i>Brassica napus</i> plants caused reduction in H <sub>2</sub> O <sub>2</sub> and lipid peroxidation levels, thus pointing towards effect of γ-Fe <sub>2</sub> O <sub>3</sub> nanoparticles on alleviating oxidative stress and improved drought resistance. γ-Fe <sub>2</sub> O <sub>3</sub> nanoparticles in capacity of fertilizer caused improvement of agronomic characters as compared to chelated iron.	Palmqvist et al., 2017
Iron oxide nanoparticles	Forest Red Gum ( <i>Eucalyptus tereticornis</i> )	Salinity	Iron oxide nanoparticles (IONPs) application resulted in management of abiotic stress in <i>Eucalyptus tereticornis</i> experiencing salt stress. Treatment of IONP (25 ppm) to the microshoots of <i>E. tereticornis</i> caused increase in superoxide dismutase, total soluble sugar and proline levels while MDA content was decreased, pointing towards the alleviation of salt stress. IONPs may up-regulate the transcript levels of salt-responsive genes encoding symporter <i>HKT1</i> , <i>NHX1</i> and <i>SOS1</i> either by efflux of Na <sup>+</sup> ions from cell or by their sequestration in vacuole during stress.	Singh et al., 2021

Iron, copper, cobalt (Co) and zinc oxide metal based NPs	Soybean ( <i>Glycine max</i> L.)	Drought	Treatment of Fe, Cu, Co and ZnO metal based NPs helped in drought resistance of soybean possibly by stimulation of drought-linked gene expression. The relative water level and biomass reduction rate were substantially improved, especially in plants with Fe-NPs-application.	Linh et al., 2020
Selenium NPs (SeNPs)	Wheat	Drought	Biosynthesized SeNPs at 30 mgL <sup>-1</sup> were optimal for enhancement in various plant morphological parameters and growth of selected drought-tolerant (V1) and drought-susceptible (V2) wheat varieties under normal and water-shortage scenarios.	Ikram et al., 2020
CeO <sub>2</sub> NPs	Cotton ( <i>Gossypium hirsutum</i> L.)	Salt	Treatment of PNC (poly acrylic acid coated nanoceria) to cotton exhibited better morphological characters, increased chlorophyll level, biomass and improved carbon assimilation rate compared to group without NP treatment, resulting in improved salt stress resistance in PNC applied cotton plants. PNC treatment caused significantly reduced MDA and H <sub>2</sub> O <sub>2</sub> levels. PNC application enabled better managed cytosolic K <sup>+</sup> / Na <sup>+</sup> homeostasis and increased tolerance to salt stress.	Liu et al., 2021

Chitosan NP encapsulated with nitric oxide (NO) donor (S-nitroso-MSA)	Maize ( <i>Zea mays</i> )	Salinity	Chitosan NP encapsulated NO donor (S-nitroso-MSA) exhibited alleviation of the impact of salinity stress such as harmful effects in photosystem II activity, chlorophyll level and growth in maize plants. The possible reason for this may be enhancing bioactivity of NO by S-nitroso-MSA nanoencapsulation.	Oliveira et al., 2016
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Ionic toxicity is also increased such as in salt stress (Ashraf, 1994). Generation of reactive oxygen or nitrogen species is also enhanced during exposure to these stresses (Chakrabarty et al., 2016). Nanoparticles-mediated stress resistance involves changes in phytohormones levels (Hao et al., 2019).

NMs mitigate abiotic stresses. During alleviation of oxidative stress experienced by plants, NMs play a role similar to antioxidative enzymes. NMs may stimulate ROS formation, thus inducing secondary signaling messengers and causing control of secondary metabolic processes at transcriptional level (Zaytseva and Neumann, 2016; Marslin et al., 2017). Generally, NMs cause a positive effect at low concentrations while at high concentrations they cause adverse effects (Agathokleous et al., 2019). Role of some important NMs in mitigating abiotic stress in important crop plants is discussed below (Table 1):

#### **Silicon Nanoparticles (SNPs)**

SNPs are very helpful in alleviation of negative effects of salinity stress on plant growth (Wang et al., 2010; Wang et al., 2011). These effects of SNPs imparting tolerance to plants under salt stress may be due to generation of a fine layer of SNPs in cell wall upon their absorption by roots (Derosa et al., 2010).

SNPs affect xylem humidity, water movement, increase turgor pressure and causing enhanced water use efficiency in plants (Wang and Naser, 1994; Rawson et al., 1998). As compared to micro-SiO<sub>2</sub>, sodium silicate (Na<sub>2</sub>SiO<sub>3</sub>), and silicic acid (H<sub>4</sub>SiO<sub>4</sub>), SiO<sub>2</sub> nanoparticles exhibited swift and improved uptake by seeds or roots of maize crop. Therefore SiO<sub>2</sub> nanoparticles due to enhanced uptake, have better utilization by plants and thus they positively affect plant growth (Suriyaprabha et al., 2012).

Silicon decreases uptake of Na<sup>+</sup> by improving K<sup>+</sup>: Na<sup>+</sup> ratio. Almutairi (2016b) investigated the role of nano-silicon in salt resistance in tomato seedlings experiencing salt stress. Nano-Si improved seed germination and growth in plants undergoing salinity stress. Effect of Si nanoparticles in plants like stimulation of gene expression and increasing activity of antioxidant enzymes, improved uptake mechanism, maintaining balance of nutrient elements, controlling synthesis of osmotically active solutes, altering gas exchange, can cause better abiotic stress resistance in plants (Liang et al., 2007; Qados, 2015).



Applying silicon as SNPs and fertilizer exerted beneficial effects on physiology and morphology of basil exposed to salt stress. Enhancement in growth, chlorophyll and proline levels in basil (*Ocimum basilicum*) was observed (Kaltah et al., 2014).

Silicon nano-particles caused enhancement of seed germination and seedling growth in lentil (*Lens culinaris* Medik.) genotypes experiencing salt stress. SiO<sub>2</sub> nano-particles help in plant's resistance for salt induced toxicity (Sabaghnia and Janmohammad, 2015). Si applied to cucumber plants resulted in enhanced activities of important antioxidant enzymes in leaves undergoing salinity stress which subsequently prevents cell membrane oxidative damage thus enhancing growth of cucumber plants. The SiO<sub>2</sub> decreases the accessibility to plasma wall of leaf cells causing loss of lipid peroxidation (Zhu et al., 2004). Silica nanoparticles decrease Na<sup>+</sup> ion concentration, possibly by decreasing Na<sup>+</sup> ion uptake by plants which helps in tolerance of salinity stress (Raven, 1983).

SNPs application enhances plant resistance toward drought in Hawthorns (*Crataegus* sp.). Physiological and biochemical responses in Hawthorns seedlings varies according to concentrations of applied silica NPs and intensity of stress through which plant is going through (Ashkavand et al., 2015).

Nano-Si alleviates heavy metal stress and improves plant growth. This is done possibly by decreasing active heavy metal ions, activation of antioxidant systems, binding and co-precipitation of toxic metals with Si, structural modifications in plants and controlling metal transport genes expression. But what type of mechanisms are followed mainly, may be possibly dependent on plant species, genotypes, types of metals to which they are exposed, growth conditions, stress duration and therefore alleviation of metal induced toxicity through Si can not be generalized (Adrees et al., 2015).

#### **Silver Nanoparticles (Ag-NPs)**

AgNPs in *Brassica juncea* (Sharma et al., 2012) resulted in enhancement in antioxidant enzymes activities (APX, guaiacol peroxidase and CAT) thus mitigating adverse effects of ROS. During molecular response of *Arabidopsis* after Ag NPs application, upregulation of 286 genes was observed while down regulation of 81 genes was observed. The upregulated genes linked with metal and oxidative stress, while downregulated genes linked with plant defence machinery and hormonal stimuli (Kaveh et al., 2013). Ag NPs treatment in rice exhibited that Ag NPs responsive proteins linked with oxidative stress response processes, Ca<sup>2+</sup> control and signaling, transcription, protein damage, cell wall biosynthesis, cell division, and programmed cell death (Mirzajani et al., 2014).

#### **Zinc oxide Nanoparticles (ZnO-NPs)**

Zinc helps plants in increasing adaptiveness of plants to resist drought stress (Cakmak, 2008). Nanozinc helps in more efficient intake of zinc and functions associated with zinc can be achieved at lesser amount. Comparable results between nano-ZnO and bulk ZnO were observed in terms of productivity and water use efficiency of sunflower plants in water shortage conditions (Seghatoleslami and Forutani, 2015). Fertilizers in nano form resulted in increased plant responses to drought compared to conventional bulk fertilization (Saxena et al., 2016). In case of *in vitro*-grown banana plants, supplementing ZnO NPs enhanced somatic embryo formation, plant regeneration and stress resistance (Helaly et al., 2014). In maize, the supplementation of nano-ZnO (400 mg L<sup>-1</sup>) resulted in enhancement of grain yield compared to control (42% higher) and zinc sulphate (ZnSO<sub>4</sub>) treated maize (15% higher); (Subbaiah et al., 2016).

The effect of nZnO, fullerene soot (FS) or nTiO<sub>2</sub> in *Arabidopsis thaliana* roots was investigated. The genes stimulated by nZnO and FS were mainly stress responsive genes (oxidative, salt, water shortage). While upon nTiO<sub>2</sub> treatment, minor alterations in gene expression linked mainly with responses to biotic and abiotic stimuli were observed (Landa et al., 2012).

#### **Titanium Oxide Nanoparticles (TiO<sub>2</sub>-NPs)**

TiO<sub>2</sub> nanoparticles have photocatalytic property and involved in oxidation-reduction reactions causing generation of superoxide anion radical and hydroxide upon light exposure (Hong et al., 2005a). Nano-TiO<sub>2</sub> can elevate water and nitrogen use efficacy in plants and stimulate SOD, POD, CAT antioxidative enzyme action in canola (Mahmoodzadeh et al., 2013). Shallan et al. (2016), reported that applying nano-TiO<sub>2</sub> or nano-SiO<sub>2</sub> through foliar route could improve drought resistance of cotton plants augmented by increase in antioxidant system capability; and enhancement of yield parameters. While, exposure to nano-TiO<sub>2</sub> can decrease Cd stress and increase Cd uptake in soybean. Possibly these responses occur due to generation of new bonds in plant tissue with Cd/nano-TiO<sub>2</sub> particles (Singh and Lee, 2016). Exposure to nano-TiO<sub>2</sub> resulted in enhanced germination rate, plant dry weight, chlorophyll generation, ribulose biphosphate carboxylase/ oxygenase activity, rate of oxygen evolution in chloroplast subsequently causing efficient photosynthesis in spinach. The enhancement of photosynthesis by nano-TiO<sub>2</sub> possibly correlated with activation of photochemical reaction of chloroplasts (Hong et al., 2005b; Zheng et al., 2005). During treatment of onion seedling with TiO<sub>2</sub> nanoparticles, there was corresponding increase in SOD activity upon enhancement in TiO<sub>2</sub> NP concentration. Seed germination and seedling growth in onion were increased at low concentration of TiO<sub>2</sub> NPs but higher concentrations have inhibitory effect. Similarly although TiO<sub>2</sub> Nanoparticles caused stimulation of amylase, CAT and POD activities, but there was an inverse relationship between increase in enzyme activities and concentration of TiO<sub>2</sub> NPs (Laware and Raskar, 2014).

#### **Iron Nanoparticles (Fe-NPs)**

Being an important micronutrient, iron is important for plant nutrition. Therefore proper absorption of iron may be crucial for drought resistance (Saxena et al., 2016). Substantial impact of Fe-NPs in plants experiencing drought stress was exhibited on boll number in a branch, seed number in a boll, 1000 seed weight and productivity (Davar et al., 2014). Exposure to Fe-NPs through foliar route exhibited drought stress alleviating effects on yield and oil content of Goldasht spring safflower. Fe-NPs also increased yield at flowering and granulation, although better results were obtained at flowering than seed generation. In plants experiencing drought stress scenarios without Fe-NPs application, same above mentioned results were not obtained (Davar et al., 2014). Kim et al. (2015), observed that treatment of nano zerovalent iron (nZVI) to *Arabidopsis thaliana* resulted in high plasma membrane H<sup>+</sup>-ATPase activity. Regarding mechanism of stomatal opening during nZVI exposure, it was exhibited that nZVI increases stomatal opening by stimulating plasma membrane H<sup>+</sup>-ATPase activation, due to which perhaps there is enhanced CO<sub>2</sub> uptake. Nanomaterials having properties similar to enzymes may be very helpful in enhancing plant's performance under abiotic stresses. Yttrium doping-stabilized  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> NPs exhibited having potential as plant fertilizer and drought stress mitigation through enzymatic activity (Palmqvist et al., 2017).

Employing iron oxide nanoparticles (IONPs) exhibited management of abiotic stress in *Eucalyptus tereticornis* undergoing high NaCl induced salinity stress. Even when plants were not experiencing stress, IONPs enhanced the shoot growth of *E. tereticornis* by increasing gene expression of antioxidant enzymes (Singh et al., 2021). Supplementation of Nano-Fe<sub>2</sub>O<sub>3</sub> (1000 mg kg<sup>-1</sup>) enhanced the gibberellic acid (GA) and zeatin riboside (ZR) levels, which improved peanut growth (Rui et al., 2016). In watermelon, nano-Fe<sub>2</sub>O<sub>3</sub> (80 mg L<sup>-1</sup>) application enhanced jasmonic acid (JA) and 12-oxo phytodienoic acid (12-OPDA) levels, resulting in improvement of stress resistance in watermelon (Kasote et al., 2019).

#### **Copper Nanoparticles (Cu-NPs)**

Cu, an essential micronutrient, has important roles in controlling plant growth and development including chlorophyll and seed production (Viera et al., 2019). Free metal Cu nanoparticles affected seed yield and quality in soybean (Quoc et al., 2014).

Application of iron, copper, cobalt, zinc oxide metal based NPs augmented drought resistance of NP-treated soybean. The drought resistance may be due to NP application induced drought-linked gene expression (Linh et al., 2020).

#### **Gold Nanoparticles (GNPs)**

Supplementation of GNPs to *B. juncea* seedlings caused increased activities of antioxidant enzymes like APX, guaiacol peroxidase (GPX), CAT and glutathione reductase (GR) coupled with enhanced H<sub>2</sub>O<sub>2</sub> and proline accumulation (Gunjan et al., 2014).

#### **Selenium Nanoparticles (Se-NPs)**

SeNPs were biosynthesized and applied through foliar route to drought-tolerant (V1) and drought-susceptible (V2) wheat plant varieties under controlled irrigation and drought conditions. SeNPs at 30 mgL<sup>-1</sup> was optimal for enhancement in various morphological parameters and growth of selected wheat varieties under normal and water-shortage scenarios. While morphological characters decreased at higher concentrations (40 mg L<sup>-1</sup>) in both wheat varieties (Ikram et al., 2020).

#### **Manganese Nanoparticles (Mn-NPs)**

Mn NPs may help plants in mitigating abiotic stresses at enhanced efficiency and decreased toxicity, in comparison to their bulk or ionic counterparts. But Mn-plant interactions, their mode of signaling and the Mn-mediated regulation mechanism need to be deciphered in detail (Ye et al., 2019).

#### **Cerium oxide Nanoparticles (CeO<sub>2</sub>-NPs)**

Upon prolonged exposure of kidney bean to 500 mg L<sup>-1</sup> nCeO<sub>2</sub>, antioxidant enzyme activities substantially decreased in roots, while the root soluble protein was enhanced. Additionally GPX activity in leaf was increased upon nCeO<sub>2</sub> application to maintain cellular homeostasis (Majumdar et al., 2014). Application of PNC (poly acrylic acid coated nanoceria) to cotton plants exhibited improved morphological parameters, enhanced chlorophyll level, biomass and improved carbon assimilation rate compared to NNP (non-nanoparticle control) group thus causing better salt stress resistance in PNC treated cotton plants (Liu et al., 2021).

#### **Chitosan NPs**

Increased production of antioxidant enzymes was a major factor in chitosan induced mitigation of adverse effect caused by drought or water shortage (Yin et al., 2008) along with enhanced root growth thus improving water absorption capacity (Zeng and Luo, 2012). Chitosan NPs encapsulated with NO donor (S-nitroso-MSA) mitigated effects of salt stress in maize (Oliveira et al., 2016). The encapsulated S-nitroso-MSA may give safety to NO donor (Seabra et al., 2014).

## Carbon Nanotubes

Multi-walled carbon nanotubes significantly impacted tomato seeds germination and seedling growth and this effect was mainly exerted through up-regulation of stress-associated gene expression (Khodakovskaya et al., 2009).

## Nanoreclaimants

Nano-reclaimants are employed in reclamation of salt-affected soils. The nano-reclaimants such as nano gypsum, nano calcium and magnesium compounds, can be easily produced, exhibit efficiency, impart improved hydraulic properties and result in soil stability (Mukhopadhyay and Kaur, 2016; Patra et al., 2016).

There are different roles of NPs in augmenting defence responses of crops to abiotic stresses. The central role of NPs through which they help in plant's adaption to stress is by activating antioxidant defence system and stimulating stress-associated genes expression (Khan et al., 2017). The plant's response to stimuli generated by environmental stress factors, is through activation of different transcellular membrane sensors, especially  $\text{Ca}^{2+}$  channels and  $\text{Ca}^{2+}$ - attaching proteins (Thapa et al., 2011). Later on transmission of these signals downstream result in gene expression changes and subsequently adaptation of crops to stress. Therefore nanomaterials help in mitigating abiotic stresses by plants mainly through activation of cellular signals because of excess ROS and/or reactive nitrogen species (RNS) production, inducing plant defence system and storage of relevant osmotically active substances, free amino acids and nutrients which help in adapting to abiotic stresses (Khan et al., 2017).

## NANOMATERIALS TOXICITY

Due to increasing use of NMs such as ENMs in recent times, instances of NMs leakage into environment has increased in recent times. Because NMs have small size which matches with the scale of size of cellular components, NMs will be having more penetrative capacity and high intensity interactions between NMs and cellular components are expected (Auffan et al., 2009). NMs toxicity has been tested in many studies including model plant *Arabidopsis thaliana* (Wang et al., 2011) and algae (He et al., 2012). Exposure of plants to NMs especially their high concentrations for a significant time period cause alterations in their morphology, physiology, biochemistry, genetics and at molecular level they result in changes in gene expression which later on affect crop growth, productivity and nutritional value (Wang et al., 2016; Zuverza-Mena et al., 2017). NMs after their uptake from soil by the plants accumulate in edible vegetative/ reproductive organs of the crops and pass on to different trophic levels with potentially serious effects on animals and humans. After their discharge into environment, NMs can remain in air, water or soil for a longer time. NMs treatment to plants undergoing stress may increase and accelerate generation of various reactive species such as ROS and RNS, thus damaging structure and functions of cell membrane, proteins, lipids and nucleic acids (e.g. DNA), affecting signaling process and causing changes in gene transcription and protein formation (Buzea et al., 2007; Khan et al., 2017).

Nanotoxicology is a subdiscipline of toxicology (Hobson, 2016), in which NMs effects on living organisms including humans and mechanism of interaction with these living organisms are investigated.

For understanding negative effects of NMs on plants, information about interaction with plants, their uptake and distribution inside plant system is required which is largely dependent upon physico-chemical characteristics of NPs and types of plant species.

Properties of the NPs like their concentration, particle size, particle shape, surface area, surface coating and functionalization, aggregation, crystal structure have profound effect on phytotoxicity induced by NMs. Type of application, experimental procedure followed, the NP treatment time, the plant's developmental phase during contact with NPs and NPs interaction with plants also affect phytotoxicity (Jeevanandam et al., 2018; Paramo et al., 2020). Formation of ROS which is dependent upon physico-chemical characteristics of NPs also significantly affects mechanism of NP induced phytotoxicity (Rui et al., 2015; Zhang et al., 2015).

### **REGULATION OF NANOMATERIALS**

Several legislative acts and rules have been framed and implemented by many governments globally to minimize or avoid risks and adverse effects caused by NMs ([http://webivadownnton.s3.amazonaws.com/877/eb/2/8482/FOE\\_Nano](http://webivadownnton.s3.amazonaws.com/877/eb/2/8482/FOE_Nano)

[BabyFormulaReport\\_13.pdf](http://www.babyformula.gov.au/~/media/1/2/12/BabyFormulaReport_13.pdf); Jeevanandam et al., 2018). However, there is no consensus globally regarding methods for toxicity testing, assessing environmental effects of NPs, manufacturing, handling and regulation of NPs. Globally accepted definition for NPs needs to be worked out properly. Globally, United States of America (USA) and European Union (EU) have strong regulatory organizations, legislative measures and guidelines to assess and control risks posed by NMs.

([http://ec.europa.eu/health/ph\\_risk/committees/04\\_scenihhr/docs/scenihhr\\_o\\_023.pdf](http://ec.europa.eu/health/ph_risk/committees/04_scenihhr/docs/scenihhr_o_023.pdf)). In US, controlling bodies like Food and Drug Administration (FDA), United States Environmental Protection Agency (USEPA) and Institute for Food and Agricultural Standards (IFAS) have started developing protocols for coping with potential risks posed by NM based products (Thomas et al., 2006). EU and Switzerland, have taken care to include regulatory aspects of NMs into their legislation for agri/feed/food, which contains methods for risk assessment for NMs use and/or legally binding definitions of "nanomaterial", and/or requirements for labeling and giving information about usage of NMs in products (Amenta et al., 2015). Many countries such as Australia, New Zealand, Canada have adopted somewhat relaxed policy and non-mandatory provisions regarding regulation of NMs in agri/feed/food. Other countries like Malaysia are employing their current governing provisions for agri/feed/food regarding control of nanotechnology (OECD, 2013a). Largely except EU, operating definitions of NMs are taken into consideration which are not legally binding. Iran, Taiwan and Thailand have devised procedures for tracing and tagging NMs based consumer products (e.g. NanoMark system), but they differ from tagging requirements in EU (Amenta et al., 2015). The Food and Agriculture Organization (FAO), United Nations and WHO are jointly working towards framing international food standards, guidelines, working codes and advisory information, which could be employed for nanotechnology-based products (<http://www.fao.org/fao-who-codexalimentarius>). Nano Science and Technology Initiative (NSTI) programme was initiated by Government of India in 2001. Later on another programme "Nano Mission" was started in 2007 (<https://dst.gov.in/scientific-programmes/mission-nano>).

Department of Science and Technology (DST) working under Central Government of India is nodal agency for implementing “Nano Mission”, while Department of Biotechnology (DBT), India has been assigned the responsibility to promote its use in different areas of life science. The DBT has been funding research activities since 2007. In November 2017, The Energy and Resources Institute (TERI), India had launched zero draft on regulation of nano-based products in agriculture area, The TERI report had stressed upon employing nanotechnology in agriculture with the purpose to decrease nutrient losses and agrochemicals and fertilizers used; through efficient distribution of functional compounds and increased crop yield through better water and nutrient management (Aggarwal, 2019). DBT, Govt. of India published guidelines for nano-based-agri input and food products (Guidelines for evaluation of nano-based agri-input and food products in India, DBT, Govt. of India, New Delhi, March, 2020). They were formed with the purpose to maintain the quality, ensuring safe and efficient use besides promoting the commercial aspects of nanotechnology-based products. The guidelines cover nano-agri-input products (NAIPs) and nano-agri-products (NAPs) beside taking into account nano composites and NMs based sensors and those requiring direct contact with crops, food and feed for data generation. According to these guidelines NAIP means NMs based agricultural formulation to be used on crop for farming while NAP includes an agricultural formulation consisting of NMs for potential use in food/feed, their supplements and nutraceutical delivery. NAIPs mainly include nano fertilizers, nanopesticides and other nano-based products for their potential employment in crop production, crop conservation, post-harvest handling and packaging. NMs can be redesigned so that their properties are changed in a way causing lower toxicity and decrease in threat they pose to environment (Maddinedi et al., 2015; Dasgupta et al., 2016). Biodegradable, non-toxic substances can be used in NMs generation so that they will be safe for employment in agriculture and NM-based agricultural products (Oomen et al., 2015). Environmental and biological monitoring can be done for assessing crop’s exposures to NMs by quantifying specific biomarkers in crops exposed to NMs (Paterson et al., 2011; Gardea-Torresdey et al., 2014). Because of small size, NMs properties are different from normal bulk materials, therefore separate set of risk assessment and regulatory measures are required for nanotechnology based products (Kookana et al., 2014). There is need for implementing regulatory measures dealing especially with NMs and NM-based products. Besides this, there is need for global agreement on a common definition of NMs for better exchange of NMs related knowledge, trade of NM-based products and improved practices for risk assessment and alleviation of NM linked hazards (OECD, 2013b).

## **CONCLUSIONS**

Nanotechnology has been recently developed from different branches of science. Nanotechnology is used in development of NMs and nanotechnology-based services are employed for enhanced crop growth and yield and mitigation of various types of abiotic stresses. NMs impart stress-resistance to plants through enhanced intake of water and nutrients mainly through roots and their movement in plants, stimulation of stress-responsive hormonal signaling pathways and expression of stress-specific genes, activating anti-oxidative enzymes, mitigating poisonous impact of various reactive oxygen species formed during stress.

Further investigations on manufacturing, absorption, movement, accumulation, biotransformation and biodegradability of NMs for secure use in agriculture and agriculture-based processes are required. Studies regarding compartmentation of NMs within subcellular organelles in plants and biomonitoring of NMs is needed. Dimensions and concentration of NMs should be optimized for reduction in their potential toxicity to crops and environment. Impact of surface operationalization and alteration of surface characters of NMs on NMs uptake and aggregation inside plants should be observed in detail. Long term toxicity of NMs on crops and environment and shifting to animals and humans by food chain should be observed in detail for proper risk-assessment and devising regulatory measures. For this crop's contact to NMs in stress conditions should be observed across many generations. Assessment of impact of NMs in mitigation of abiotic stress should be done in actual field conditions where crops are simultaneously exposed to different abiotic stresses or through simulating actual field conditions, for a better idea of NMs induced stress-resistance mechanism. Non-toxic, biodegradable NMs can be developed for safe agricultural applications. The mechanism of stress-resistance induction in crops by NMs should be investigated at molecular level including detailed studies at transcriptomics and proteomics levels. Alterations in secondary metabolites profile in crops under stress with NMs treatment should be studied. Effective biomonitoring methods for NMs should be developed and implemented globally for their safe use in agriculture. Standardized risk-assessment protocols should be developed at global level through joint efforts of both government and private organizations. There should be consensus globally regarding definitions of NMs and nanotechnology for easy coordination of NM-based research and devising policies and people should be made aware about both positive and negative aspects of use of NMs and NM-based products.

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Corresponding Author: Dr. Mohd. Zahid Rizvi, Department of Botany, Shia Post Graduate College, Sitapur Road, Lucknow-226020, Uttar Pradesh, India  
Email: [zahid682001@gmail.com](mailto:zahid682001@gmail.com)