Turkish Journal of Range and Forage Science, 2021, 2(2): 52-62
doi: 10.51801/turkjrfs.954843

Review

Turkish Journal of Range and Forage Science

https://dergipark.gov.tr/tr/pub/turkjrfs

The Effect of Nanoparticle Applications on Plants under Some Stress Conditions

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A R T I C L E I N F O

Received *20/06/2021*
Accepted *30/08/2021*

Keywords:
Drought
Heavy metal stress
Nanoparticles
Plant growth
Salinity

A B S T R A C T

Plants are exposed to various abiotic stresses such as drought, salinity, high temperature, flooding and heavy metal stress. These stress factors have a significant negative effect on plant growth and yield and cause economic losses. Therefore, new approaches such as nanotechnology are used to reduce the harmful effects of these stresses on plants. Agricultural nanotechnology aims to improve sustainability in agriculture, to use water effectively and to protect against plant diseases, to eliminate environmental pollution and the effects of abiotic stress factors. Nanoparticles eliminate nutrient deficiencies in plants, increase the tolerance of plants to stress conditions by enabling the enzyme activities and the adhesion of bacteria that promote plant growth to the roots under abiotic stress conditions. In this review, the role of nanoparticles in ameliorating adverse effects on plants exposed to abiotic stress conditions will be emphasized.

1. Introduction

Unsuitable environmental conditions are defined as stress, and many abiotic and biotic environmental factors cause stress in plants. Abiotic stress conditions such as high or low temperature, waterlogging, drought, salinity, heavy metals and ultraviolet radiation adversely affect many morphological (plant height, leaf area, shoot length, root length etc.), physiological (net photosynthesis, PSII efficiency (*Fv/Fm*), stomatal conductance etc.) and biochemical (proline, soluble protein, soluble sugar content etc.) processes that directly affect plant growth.

Abiotic stress

As the world population increases, abiotic stress conditions will continue to be a greater challenge for crop production. During stress, physiological and biochemical changes occur in plant cells and growth and development decrease and as a result, plant yield is adversely affected. In order to achieve a sustainable production under changing climatic and stress conditions it is necessary to develop varieties resistant to stress conditions or to apply nanotechnology and other climate sensitive agricultural technologies.

Drought and heat stress are increasing seriously as a result of climate change and are among the abiotic stress factors that negatively affect crop production. In addition waterlogging, salinity and heavy metal stress are among the environmental factors that limit crop production almost all over the world (Ye et al., 2019). Drought affects the physiological and biochemical processes of plants

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especially the synthesis and accumulation of secondary metabolites. In arid conditions, some physiological changes occur in the plant, and some biochemical processes such as antioxidant enzymes and phenolic content are affected. Signals from roots to leaves are transmitted via xylem vascular bundles (Afshari et al., 2021). Turgor loss is observed in plants with drought stress. Dehydration increases in the protoplasm. The damage to the plant causes irreversible problems in cellular metabolism and the growth of the plant slows down. Due to water loss, cellular metabolism, ion accumulation, membrane structure integrity and protein structures are disrupted in the plant. Leaf size decreases, photosynthesis products are reduced, enzyme activity and amount are also affected. Drought also causes the formation of Reactive Oxygen Species (ROS) in plants and in connection with this it causes oxidative stress (Cruz de Carvalho, 2008).

Salinity is a common abiotic stress factor that causes significant reductions in plant growth. Soil salinity can affect seed germination by creating an osmotic potential that prevents water uptake outside the seeds or by Na^+ and Cl^- ions (Tavakkoli et al., 2010). NaCl causes oxidative damage in different legumes, leading to a significant decrease in different growth parameters, seed nutrient quality and nodulation (Hernandez et al., 2000; Ahmad et al., 2008). To reduce and repair damage induced by oxidative stress plants have developed a set of antioxidant defense mechanisms both enzymatic and non-enzymatic. While ascorbate and carotenoids are two important non-enzymatic defense mechanisms against salinity, proline is the most discussed osmoregulatory substance under stress (Anoop et al., 2003).

Heavy metals include iron, zinc, manganese, copper, nickel, molybdenum and cobalt which are essential for plant nutrition and non-essential elements chromium, cadmium, mercury, and lead. All these elements are highly toxic to plants at high concentrations (White and Pongrac, 2017).

Toxic levels of heavy metals adversely affect various metabolic processes such as degradation or displacement of protein structures resulting from the formation of bonds between heavy metals and sulfhydryl groups (Hall, 2002), disruption of the integrity of the cytoplasmic membrane (Farid et al., 2013), causes suppression of vital events such as photosynthesis, respiration and enzymatic activity (Hossain et al., 2012).

Nanoparticles

Nanoparticles (NPs) are microscopic particles with sizes in the range of 1-100 nm (Khan and Upadhyaya, 2019). Most of the NPs that enter the cell from above-ground organs (cuticle, epidermis, stoma, hydatid and other openings) or underground organs (root tips, cortex, lateral root, wounds and other openings) have a variety of physiological and morphological effects on plants. The effects of these compounds vary depending on the plant species, growth period and growing conditions, application method, dose and exposure time (Dietz and Herth, 2011; Rizwan et al., 2017).

NPs which enter through stomata are carried within the plant by the phloem. NPs are transported as a result of pressure differences between leaves and roots depending on mass flow or pressure flow theory (Yıldız, 2018).

The way the nanoparticles are taken to the plant causes differences in many processes such as germination, antioxidant activity, macro and micro-nutrients, chlorophyll content, chloroplast number and photosynthesis in plants (Cinisli et al., 2019). Nanoparticle applications also change intra-root signals by affecting ethylene production of *Arabidopsis* roots (Syu et al., 2014).

Nanoparticles penetrate the cell membrane and cell wall. They reach the epidermis, xylem, central cylinder and finally the leaves (Tripathi et al., 2017). Before reaching the central cylinder, nanoparticles are passively transported in the endodermis (Judy et al., 2012). The uptake mechanism of nanoparticles is mostly through active transport.

Nanoparticles enter the plant root through osmotic pressure, capillary force and cell wall pores through plasmodesmatal connections or symplastic ways. Nanoparticles bind to the carrier protein via ion channels, aquaporin and endocytosis, form new pores and enter the plant cell. After nanoparticles enter the plant cell, they can be transported from one cell to another via plasmodesmata via apoplast or symplast (Usman et al., 2020; Fig. 1). The entry of nanoparticles through the cell wall depends on the pore size of the cell wall. Small-sized nanoparticles easily pass through the cell wall (Fleischer et al., 1999), while larger nanoparticles pass through stomata, hydathode and stigmas (Hossain et al., 2016).

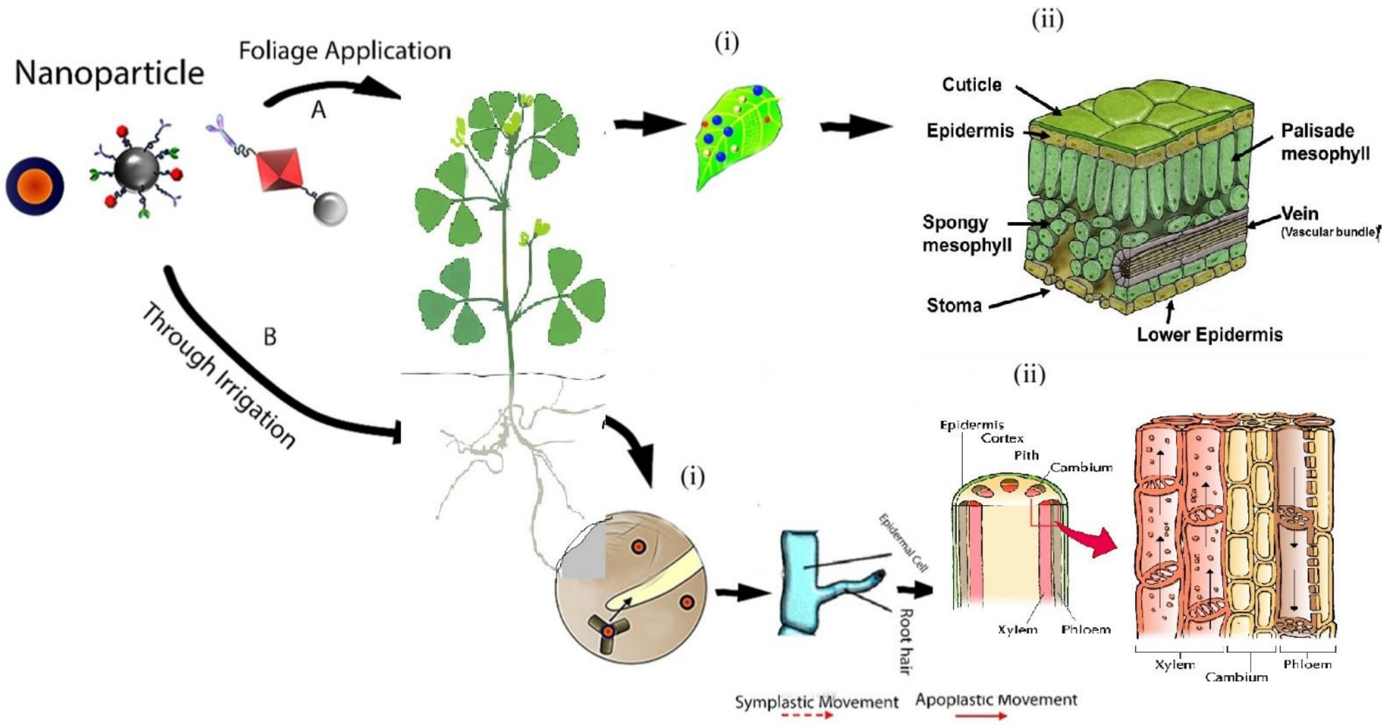

Fig 1. Uptake and transport mechanism of nanoparticles via leaves and roots. (A) uptake of nanomaterials by foliar application (i) entry of nanomaterials through leaf cuticle (ii) entry from epidermis layer to palisade and sponge parenchyma and transition to vascular cambium (B) uptake of nanomaterials by irrigation by plant roots (i) entry of nanomaterials through root hairs (ii) Access to xylem and phloem via epidermis and cortex (apoplastic and symplastic) (Usman et al., 2020).

Transport of nanoparticles occurs through stomata when the particle size is 40 nm or larger (Eichert et al., 2008). These nanoparticles accumulate in the stoma instead of the vascular cambium and are then transported to different parts of the plant via the phloem (Tripathi et al., 2017). Nanoparticles enter through parenchymatous intercellular spaces in the seed coat (Lee et al., 2010). However, in the seed coat, aquaporins play a role in regulating the entry of nanoparticles (Abu-Hamdah et al., 2004).

With the increase in nanotechnological applications, the use of nanomaterials with a high surface-to-volume ratio has also increased. The functions and usage areas of nanomaterials differ according to the size and composition of the nanoparticles (Tunca, 2015). When nanomaterials are used as fertilizers, the plant provides nutrients slowly, very small amounts are sufficient compared to chemical fertilizers, and they have a positive effect on the plant and nature by reducing the environmental risks caused by chemical fertilizers (Cinisli et al., 2019; Usman et al., 2020).

The environment and human health are adversely affected due to the unconscious and high doses of chemical pesticides and fertilizers in agriculture to increase the yield of plants. Therefore, it has become a necessity to replace pesticide and fertilizer applications with nanopesticides and nano fertilizers in order to reduce the use of chemical fertilizers, increase plant yield, and also support agricultural development (Bratovcic et al., 2021). Research into nanotechnology applications in agriculture has become increasingly popular in the last decade, and especially new nanoagrochemicals called "nanopesticides" and "nanofertilizers" have been the focus of attention (Kah, 2015).

Nano-fertilizers have very small dimensions ranging from 30 to 40 nm, can pass through stomata very easily, can hold many ions and are released slowly to meet the nutrients needed by the plant (Bal, 2019; Cinisli et al., 2019).

The effect of nano fertilizers on plant development and metabolism is due to the fact that they make plants resistant to biotic-abiotic stress conditions and diseases in variable and severe atmospheric conditions, as well as their effect on enzymatic and hormonal levels (Cinisli et al., 2019).

Nanopesticides can be synthesized by physical, chemical or biological methods. Nanopesticides and nanoformulations such as Ag, Cu, SiO_2, ZnO show better broad spectrum pesticide efficacy compared to conventional pesticides used. For this reason nanopesticides have a positive influence on the control of plant pests and diseases (Chhipa, 2017).

In recent years, chitosan-metal oxide nanoparticles have been used to ensure that the fertilizers to be applied to the plants are taken up more effectively by the plants. Chitosan application increases nitrate reductase, glutamine synthetase and protease enzyme activities in N metabolism and thus affects plant growth and development (Bal, 2019).

Zinc oxide nanoparticles increased germination percentage and improved seedling growth in peanut and corn plants (Prasad et al., 2012; Singh et al., 2017). While root length shortened in corn seedlings treated with 2000 mg/L 60 nm Al NP for 5 days, there was no adverse effect on *Raphanus sativus, Brassica napus, Cucumis sativus, Lolium perenne* and *Lactuca sativa* (Yang and Watts, 2005). Application of 2000 mg/L Zn nanoparticles significantly inhibited root growth in maize and stopped root growth of *Cucumis sativus, Glycine max, Brassica oleracea* and *Daucus carota* (Lin and Zhing, 2007).

Few studies have addressed the effect of nanoparticles on seed germination and seedling growth by seed pretreatment in forage and medicinal plants. In general, it has been observed that nanoparticle application to seeds increases seed germination, seedling growth and development, seedling viability and emergence rate (Khalaki et al., 2021).

Seed germination, root and shoot length, fresh and dry weight values of *Agropyron elongatum* were positively affected by SiO_2 nanoparticle application (Azimi et al., 2014). Abbasi Khalaki et al. (2016) emphasized that AgNPs increased the germination rate, root and shoot length, fresh and dry weight, average germination time and vitality index in *Thymus kotschyanus* plant. Similarly it has been reported that silver nanoparticles increase the germination rate in *Pennisetum glaucum* (Parveen and Rao, 2015) and *Festuca ovina* (Abbasi Khalaki et al., 2019a). Amooaghaie et al. (2015) emphasized that Ag NPs negatively affect the germination of *Brassica nigra*. In addition, it has been reported that *Medicago sativa* negatively affects shoot length, *Ocimum basilicum* root length, root and shoot dry weight, and shoot and root length in *Linum usitatissimum, Lolium perenne* and *Hordeum vulgare* (Al-Temsah and Joner, 2010; Ramezani et al., 2014; Yosefzaei et al., 2016).

SiO_2 nanoparticles applied to *Onobrychis sativa* increased shoot length, while TiO_2 nanoparticles increased germination time and percentage (Moameri et al., 2018a). Wang et al. (2011) found that Fe_2O_3 nanoparticles increased the germination of *Lolium perenne*. FeO NPs caused a decrease in mycorrhizal biomass, root and shoot length in *Trifolium repens* (Feng et al., 2013), *Satureja hortensis* (Peyvandi et al., 2011a), *Lolium perenne* and *Hordeum vulgare* (El-Temsah and Joner, 2010).

Feizi et al. (2013) observed that TiO_2 NPs positively affected germination in *Foeniculum vulgare*, Dehkourdi and Mosavi (2013) also reported same results about *Petroselinum crispum*, Ag NPs were found to increase shoot length and chlorophyll content in *Brassica juncea* and *Sorghum bicolor* (Namasivayam and Chitrakala, 2011; Sharma et al., 2012). It was stated that the root growth of *Thymus kotschyanus* and *Alopecurus textilis* was positively affected by SiO_2 NP application (Abbasi Khalaki, 2019a; 2019b).

Similarly, SiO_2 application to *Medicago sativa* increased plant height, number of tillers, yield, fresh and dry weight, chlorophyll and carotenoid content (Ma and Yamaji, 2006; Zmeeva et al., 2017). Siddiqui et al. (2007) reported that SiO_2 increased leaf fresh and dry weight and chlorophyll content in *Ocimum basilicum*. SiO_2 NP application was found to affect shoot and root growth negatively in *Sorghum bicolor*, *Stipa hohenackeriana* and *Secale montanum* plants (Lee et al., 2012; Moameri et al., 2018b; Moameri and Abbasi Khalaki, 2019).

ZNO NPs increased plant biomass, shoot and root length and chlorophyll content (Peyvandi et al., 2011b; Wang et al., 2011; Raliya and Tarafdar, 2013; Najaf Disfani et al., 2016; García-López et al., 2018; Yuan et al. 2018). TiO_2 NPs increased the essential oil content and yield in medicinal plants (Ahmad et al., 2018; Fazeli-Nasab et al., 2018). CuO NPs adversely affected the morphology, physiology and biochemistry of *Hordeum vulgare, Lolium perenne, Triticum aestivum* and *Medicago sativa* (Lee et al., 2008; Atha et al. 2012; Ramezani et al., 2014; Shaw et al., 2014; Hong et al., 2016).

Nanoparticles under salinity conditions

Nanoparticle applications gain importance in order to improve the harmful effects of abiotic stress conditions on plants, to obtain the desired yield and quality, and to increase the resistance of plants to salinity.

At the germination stage, the application of Ag nanoparticles to *Lathyrus sativus* L. plants under salt stress improved germination percentage, shoot and root length, and seedling fresh and dry weight. Therefore, it has been reported that Ag nanoparticles are important for osmotic regulation in *Lathyrus sativus* L. under salt stress, Ag application reduces the negative effects of salinity and the toxic effects of salt stress on the plant (Hojjat, 2019). Noman et al. (2020) stated that the application of Cu-nanoparticle to the soil reduces oxidative stress in wheat and significantly increases plant growth and yield. The application of nanoparticles in wheat not only increases plant growth, but also improves germination performance under salt stress conditions (Eshi et al., 2016). Pre-application of Ag-nanoparticles to wheat seeds changed antioxidant enzyme activities, reduced oxidative damage and increased tolerance to salt stress (Kashyap et al., 2015). Zinc oxide (ZnO) nanoparticles increased dry weight in sunflower under salt stress conditions (Torabian et al., 2016).

CeO NPs (100 and 200 mg/kg) improved the physiological parameters of *Brassica napus* L under salt stress (100 mM NaCl). Similarly, it was observed that the application of CeO nanoparticles to the canola under salt stress conditions increased the plant biomass (Rossi et al., 2016). The application of silver nanoparticles to basil seeds under salt stress conditions increased the germination of the seeds (Darvishzadeh et al., 2015; Hojjat and Kamvab, 2017).

In salt stress conditions, the application of silver nanoparticles to *Satureja hortensis* L. plants increased the plants' tolerance to salt stress, along with reduced germination rate and plant shoot length due to salt stress (Nejatzadeh, 2021). The application of silver nanoparticles to cumin plants under salt stress significantly increased the salt tolerance of the plants (Ekhtiyari and Moraghebi, 2012). Askary et al. (2017) reported that Fe_3O_4 nanoparticles have a protective role against oxidative stress caused by NaCl in mint.

Nanoparticles under drought conditions

Drought is one of the abiotic stresses that significantly limits crop production. Therefore, nanoparticle application is effective in mitigating the effects of drought on plants due to its many positive effects such as increasing antioxidant enzyme activity, improving phytohormone levels and effecting on physiological properties.

Application of analcite nanoparticles to soil in hot and dry conditions has been shown to promote germination and plant growth in wheat (Hossain et al., 2021). Application of ZnO NPs to soybean seeds in arid conditions increased the percentage of germination in seeds (Sedghi et al., 2013). The application of Cu and Zn NP to wheat plants under drought stress increased antioxidant enzyme activity and relative moisture content, decreased thiobarbituric acid, reagent accumulation, stabilized the photosynthetic pigment in leaves and alleviated the effects of stress (Taran et al., 2017). Under drought stress, SiO_2 nanoparticle application increased shoot length and relative water content in barley but reduced superoxide radical formation and membrane damage (Yıldız, 2018).

Jaberzadeh et al. (2013) emphasized that foliar application of titanium dioxide (TiO_2) NPs to wheat in arid conditions was effective in overcoming the yield reduction caused by drought stress. Application of copper nanoparticles to maize under arid conditions increased leaf water content, plant biomass, anthocyanin, chlorophyll and carotenoid content (van Nguyen et al., 2020). Ashkavand et al. (2015) emphasized that the application of SiO_2 nanoparticles to hawthorn grown under drought stress conditions led to a decrease in photosynthesis and stomatal conductivity in plants. However, it has been determined that silicon nanoparticles improve the effects of drought stress in bananas (Mahmoud et al., 2020). In moderate drought conditions, foliar application of silicon nanoparticles to coriander resulted in optimum antioxidant capacity and essential oil yield (Afshari et al., 2021).

Shallan et al. (2016) emphasized that the foliar application of SiO_2 and TiO_2 nanoparticles reduced their negative effects on cotton plants in arid conditions. The application of silicon nanoparticles to the soil decreased the harmful effects of drought by increasing the relative moisture content of the chickpea (Güney et al., 2007).

Drought stress resulted in greater enhancement of the negative effect of Cd in wheat, while the application of ZnO NPs ameliorated both Cd and drought stress (Khan et al., 2019).

Nanoparticles under heavy metal stress

Under heavy metal stress conditions, soil or foliar applications of nanoparticles eliminate the negative effects of stress, improve plant growth and photosynthesis, and reduce oxidative stress-induced toxicity. Therefore, the application of nanoparticles appears to have a potential role in remediation of heavy metal-contaminated environments.

In heavy metal stress conditions nanoparticle application to plants helps to decrease heavy metal concentration in soil regulate the expression of heavy metal transfer genes in plants, increase plant antioxidant systems, improve physiological functions and stimulate the production of protective agents (e.g. root secretions, phytochelatin and organic acids) (Rui, 2021). Application of silicon nanoparticles to maize plants under arsenic stress conditions improved the reduction in total chlorophyll, carotenoid and total protein content. In addition, it was revealed that the negative effects of arsenic stress on the maximum quantum efficiency, photochemical quenching and non-photochemical quenching of FS II decreased with the application of silicon nanoparticles (Tripathi et al., 2019). Soil application of TiO_2 NPs effectively limited Cd toxicity by increasing physiological parameters and photosynthesis rate in soybean. Therefore, TiO_2 NPs are of great importance in mitigating the effects of heavy metal-induced oxidative stress (Singh and Lee, 2016). The activities of enzymes such as superoxide dismutase, ascorbate peroxidase increased and the effects of oxidative stress decreased in pea seedlings under chromium stress with silica nanoparticles (Tripathi et al., 2015).

de Sousa et al. (2019) revealed that Si NPs alleviate Al toxicity by activating the antioxidant defense system in maize plant. Konate et al. (2017) emphasized the protective role of Fe_3O_4 nanoparticles against cadmium-induced oxidative stress in wheat.

Zhang (2019) reported that the foliar application of Se NPs to Chinese cabbage under Cd stress increased the biomass, plant height, leaf chlorophyll content, SOD and GSH-Px content of Chinese cabbage, while the Cd content and MDA content of the leaves decreased. Similarly, it has been emphasized that silicon nanoparticles reduce Cd stress in rice (Wang et al., 2015). Combined application of foliar ZnO NP and soil biochar to plants under cadmium stress was more effective against Cd stress (Ali et al., 2019; Rizwan et al., 2019a).

Under Cd stress conditions, application of FeO NPs to wheat, soil and foliage both decreased leaf electrolyte leakage rate, Cd content in grains, as well as increased antioxidant enzyme activity and dry weight of wheat. In addition, foliar application of Fe NPs is more preferred than soil application. This is due to many factors such as the absorption of Fe in the soil, pH and interaction with other minerals during absorption. Co-application of Fe nanoparticles with biochar (Rizwan et al., 2019a) alleviated the effects of Cd stress in rice (Hussain et al., 2019).

20 mg/L Fe_3O_4 nanoparticle application reduced cadmium accumulation in tomato plant and improved cadmium toxicity by increasing nutrient intake (Rahmatizadeh et al., 2019).

Nano-fertilizers aganist commercial fertilizers

Nanoparticles improve the solubility and distribution of insoluble nutrients in the soil, increase the efficiency of fertilizers in plant production and the uptake of nutrients in the soil thereby saving fertilizer. Nanofertilizers also prolong the effectiveness of fertilizers and reduce losses through washing. On the other hand in commercial fertilizers, there is less benefit for plants due to the large particle size and less solubility. As a result of high fertilizer release toxicity occurs, which disrupts the ecological balance of the soil. After some of the fertilizer is used by the plants the remaining part turns into insoluble salts in the soil. In addition high losses occur due to washing.

2. Results

Recently, nanotechnological studies have come to the fore in coping with stress conditions in crop production. Nanoparticles promote physiological and biochemical processes to increase plant growth and development under stress conditions. Plants on the other hand give different responses depending on the size, shape, application method and physicochemical properties of nanoparticles. The application of the nutrients needed by the plant in the form of nanomaterials enables the plant to benefit from the fertilizer at the maximum level. The application of nanoparticles increases the adhesion in the soil due to their large surface areas and prevents them from being easily washed away. Thus, the cost of chemical fertilizers and environmental pollution will be prevented. The effectiveness of NPs even at very low concentrations and the effect on plants varies depending on the species and dose. Compared to conventional fertilizers, they appear as an alternative solution to overcome the problems related to abiotic stress in plants because they are more efficient and effective.

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