ABSTRACT

Soil salinity is one of the major environmental stresses that imparts drastic effects on plant growth and development. The adverse effects of salinity affect the overall plant health, resulting in impaired physiological and biochemical attributes. Poor health ultimately causes a loss in plant production rate and nutritional values. To overcome the negative effects of salinity and to improve the yield of crop plants, major emphasis has been given to the application of several fertilizers, pesticides, and fungicides. Moreover, the development of genetically modified crops has been advocated for the development of salt-tolerant crop plants. However, these have some drawbacks, such as genetic modification being time-consuming and cost-consuming and applying chemical fertilizers hampering soil fertility. Moreover, the efficiency of fertilizers, considered important for improving crop yield, reduces soil fertility due to over-application, resulting in residual toxicity on crop plants. In this context, in the last decade, nanotechnology has emerged as an alternative strategy for the betterment of plant health as well as environmental stress alleviation. The idea behind using nanoparticles in crop improvement was to improve the efficiency of the bulk materials already used as agrochemicals. Because of having a high surface area to volume ratio, the nanomaterials facilitate easy uptake and render them more interactive with the cellular active components, thereby amplifying their efficiency.

Though silica is not an essential element in plants, its deficiency creates various problems; thus, it is considered a semi-essential element. The application of silica nanoparticles (SiNPs) has excellent potential for crop improvement because it increases silica availability to plants. The synthesis of any nanoparticles demands an optimization process through which we can understand the ratios of components that should be used to get the

actual shape, size, and surface charge. In this purview, the second chapter presents the synthesis, optimization, and characterization of SiNPs. SiNPs were optimized by varying the three important ingredients (TEOS, ethanol, and ammonia) through 'Design Expert 13' based 'mixture design', which revealed that the size of the SiNPs can be modified but not the surface charge. Using mixture design, we successfully obtained ~50 nm-sized SiNPs, which was confirmed through several analytical techniques.

To study the potential of SiNPs in salinity stress alleviation, four concentrations of NaCl (for seedling and vegetative stage – 0, 100, 200, and 300 mM; for reproductive stage – 0, 200, 400, and 600 mM) in combination with four concentrations of SiNPs (0, 1, 5, and 10 g/L) were applied in two legume crops – lentil, and soybean, respectively (Chapters 3 and 4). The results depicted that both plants had better germination attributes under salinity after the application of SiNPs. In the seedling and vegetative stages, SiNPs were found to improve the plant's health under salinity through improved plant height, relative water content, photosynthetic pigments, ionic and osmotic balance, antioxidant defense (enzymatic and/ non-enzymatic), membrane stability and reduced ROS accumulation. Similarly, in the reproductive stage of both legumes, applying SiNPs was also observed to reduce the salinity stress impacts through improved plant health, yield, and nutritional aspects of the seeds.

The use of bare SiNPs has already been proven to have beneficial attributes for crop plants under salinity. However, very little research has focused on applying functionalized nanoparticles for crop improvement under environmental stresses. Surface functionalization of these nanoparticles with bioactive molecules has the potential to increase their efficiency in this regard. Thus, in chapters 5 and 6, the SiNPs surface was functionalized with two important sugars/osmolytes – glucose (GSiNPs) and trehalose (TSiNPs), respectively. Both surface functionalizations were confirmed using several analytical techniques, including

FTIR, XRD, DLS with zeta potential, UV-visible spectroscopy, SEM, EDS, TEM, and AFM. Applying both GSiNPs and TSiNPs improved the health of lentil and soybean seedlings through improved height, photosynthetic pigments, ion balance, and antioxidant defense under salinity stress. In both plants, the improvement was found to be more profound with the use of GSiNPs and TSiNPs compared to the bare SiNPs. The bioassay experiments also proved that the functionalization of the SiNPs surface with glucose/trehalose increased the bioavailability and uptake of both silica and sugars in the seedlings. Thus, these studies can provide ample evidence of the greater efficacy of surface-functionalized nanoparticles in palliating salinity stress in plants.

Apart from the morphological, physiological, and biochemical analyses, we performed the molecular and genetic background of the beneficial effects of surface functionalized SiNPs (TSiNPs) by performing the whole transcriptome analysis (RNA-Seq). The results revealed that the TSiNPs have regulatory roles on various genetic expression, which modulates various protein expressions, pathway functions, cellular components, molecular functions, and biological processes to confer progressive health benefits for lentil seedlings under salinity. More specifically, some of the genes involved in the providence of salinity stress tolerance (Sodium hydrogen exchanger, Potassium transporter, Cytochrome b6, sugar transporter, and ABC transporter G family member) were expressed highly in the presence of TSiNPs.

Along with their beneficial effects on crop improvement, various nanoparticles have also been found to have toxic effects on plants, animals, and environmental elements. So before proceeding to the filed application and commercialization, the SiNPs must also be checked for their toxicity attributes. In this purview, the cytotoxicity and genotoxicity of the synthesized SiNPs were evaluated on the *Allium cepa* root tip, considering their applied concentration and size. Further, the toxicity effects of SiNPs were attempted for attenuation using surface functionalization with sugar molecules – GSiNPs and TSiNPs. The results showed that the SiNPs showed toxic effects after the 100 g/L concentration, and the 30 nm and 100 nm sized SiNPs showed more toxic effects than the 50 nm-sized nanoparticles. Further, the GSiNPs and TSiNPs were found to reduce the toxicity level of SiNPs even at higher concentrations (> 100 g/L). A toxicity study was also performed on soil microflora, and the results showed that the SiNPs, GSiNPs, and TSiNPs improved the soil microbial communities (mixed soil bacteria, phosphate solubilizers, nitrogen fixers, and silica solubilizers) in control (0 mM NaCl) as well as 300 mM NaCl stress conditions.

Finally, the SiNPs, GSiNPs and TSiNPs were developed into nanoformulations (slow-release type) using sodium alginate as internalizing polymers. The results of Chapter 9 revealed that the alginate internalization makes the silica and/or sugar release from SiNPs, GSiNPs, and TSiNPs at a slower rate, considering the effect of incubation time, pH, and temperature. These nanoformulations also improved the seedling growth even better than the bare and functionalized SiNPs, even in the presence of NaCl stress. However, the cost of production at the laboratory scale is relatively high, and our future emphasis is to make these nanoformulations cheaper so that they can handed over to the farmers for field application.

Mahima Misti Sarkar

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