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Research article

Nitric oxide alleviates silver nanoparticles (AgNps)-induced phytotoxicity in *Pisum sativum* seedlings☆

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ABSTRACT

Understanding the adverse impact of nanoparticles in crop plants has emerged as one of the most interesting fields of plant research. Therefore, this study has been conducted to investigate the impact of silver nanoparticles (AgNps) on *Pisium sativum* seedlings. Besides this, we have also tested whether nitric oxide (NO) is capable of reducing toxicity of AgNps or not. NO has been found as one of the most fascinating molecules, capable of enhancing plant tolerance to different environmental stresses. The results of the present study showed that AgNps treatments (1000 μM and 3000 μM) significantly declined growth parameters, photosynthetic pigments and chlorophyll fluorescence of pea seedlings, which could be correlated with increased accumulation of Ag in root and shoot of pea seedlings. In contrast, addition of SNP (100 μM; a donor of NO) successfully ameliorated AgNp-induced adverse effects on these parameters as it reduced accumulation of Ag and repaired damaged tissues. Levels of oxidative stress markers (SOR, H_2O_2 and MDA) were enhanced while their levels significantly reduced under SNP addition. AgNps (1000 μM and 3000 μM) significantly stimulated the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) while inhibited activities of glutathione reductase (GR) and dehydroascorbate reductase (DHAR). AgNps also considerably declined the total ascorbate and glutathione contents and severely damaged leaf and root anatomical structures. On the other hand, addition of SNP further increased the level of SOD, APX, GR and DHAR and significantly increased the decreased levels of total ascorbate and glutathione contents, and repaired anatomical structures. In conclusion, this study suggests that AgNps treatments adversely decreased growth, pigments and photosynthesis due to enhanced level of Ag and oxidative stress. However, SNP addition successfully ameliorates adverse impact of AgNps on pea seedlings by regulating the Ag uptake, antioxidant system, oxidative stress and anatomical structures of root and shoot.

Abbreviations: F_0, minimal fluorescence; F_v/F_m, maximum photochemical efficiency of PS II; F_v/F_0, the activity of PS II; qP, photochemical quenching; NPQ, non-photochemical quenching; APX, ascorbate peroxidase; SOD, superoxide dismutase; GR, glutathione reductase; DHAR, dehydroascorbate reductase; ROS, reactive oxygen species; SOR, superoxide radical; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; LE, lower epidermis; UE, upper epidermis; VB, vascular bundle; PI, palisade cells; SP, spongy parenchyma; EEC, elongated epidermal cells; EP, epidermis; RH, root hairs; VT, vascular tissue; XYL, xylem; PHL, phloem.

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1. Introduction

In the present time, nanotechnology has emerged as one of the most capable and significant techniques, which has manifold roles not only in the science but in prospering human life as well (Ma et al., 2010; Nair et al., 2010; Yin et al., 2012; Lee et al., 2012; Geisler-Lee et al., 2014; Tripathi et al., 2015). The development of nanotechnology and useful nanomaterial have not only resolved many questions and problems of various disciplines of science, but also created some problems, which would be faced by the future generations (Ma et al., 2010; Nair et al., 2010; Yin et al., 2012; Lee et al., 2012; Geisler-Lee et al., 2014; Tripathi et al., 2015). Nanoparticles (NPs) are supposed to be more active than their parent compounds because they have large surface area and novel physical and chemical properties, which are by and large not found in the bulk particles of the same material (Stampoulis et al., 2009; Amooaghaie et al., 2015). In coming year, release of NPs in the environment is supposed be enhanced because of their industrial production. Release of NPs in the environment by various industries may directly make contact with the soil, water bodies, microorganisms and plants, which may severely harm their natural metabolic systems and cause various toxic impacts. This problem further becomes more dangerous in those countries where the proper regulatory legislations regarding nanomaterial have not been implemented by the government (Ma et al., 2010; Nair et al., 2010; Yin et al., 2012; Lee et al., 2012; Geisler-Lee et al., 2014).

There are many NPs, which are beneficial for various purposes but when they come into the contact of plants cause severe reduction in their growth and development. Among these, silver nanoparticles (AgNPs) are one of them. It has been well documented that AgNPs are being used very frequently in consumer product inventories. Fabrega et al. (2011) have demonstrated that presently AgNPs are used in more than 250 products because of their well known anti-microbial properties and utilities in individual-care products, electronics, food services, energy production, building materials, medicines, medical instruments, healthcare, textiles and environmental remediation (Park et al., 2010; Amooaghaie et al., 2015). Further, there are many studies which show that AgNps are highly toxic to plants, fish, algae, bacteria, human and other organisms (Pal et al., 2007; Jiang et al., 2008; Asha Rani et al., 2009; Miao et al., 2010). However, there are still very limited studies, which could show actual mechanisms of AgNps toxicity in plants, so it has been remain unexplored to understand the real phenomenon of AgNps toxicity (Asha Rani et al., 2009; Miao et al., 2010). A report has demonstrated that the rising production of commercial AgNps might have imposed the harmful effects on ecosystems (Kaegi et al., 2005). Thus, AgNps or Ag^+ may pollute agricultural settings, with potential impacts on plant health, growth and productivity (Navarro et al., 2008; Lee et al., 2012; Dimkpa et al., 2013). In spite of these facts about the risks of AgNPs in the plant system, there are no studies related to alleviation of AgNps-induced toxicity in plant thus, making this scientific question important to discover a technique against the AgNp-induced toxicity in plants.

There are ample studies, which indicated that nitric oxide (in the form of SNP) alleviates various type of abiotic stresses including heavy metal stress in plants (Song et al., 2006; Zhang et al., 2009; Singh et al., 2013; Chen et al., 2015). As NO is a gaseous free radical, which has been accounted as one of the most significant upbeat regulators that performs as an inter-as well as intracellular signaling molecule under stress (Song et al., 2006; Arasimowics and Floryszak-Wieczorek, 2007; Shi et al., 2007; Zhang et al., 2009; Xiong et al., 2010; Xu et al., 2010; Singh et al., 2013; Amooaghaie et al., 2015). Beside ample evidences in literature related to NO-mediated alleviation of abiotic stresses, there is not much work carried out dealing with NPs and NO interaction in plants. However recently, Chen et al. (2015) have shown that NO alleviates zinc oxide nanoparticles toxicity in rice seedlings. Thus, it is important to investigate whether NO is really capable of alleviating the toxicity of other nanoparticles including AgNps in plants.

Therefore, this study was designed to investigate (i) AgNps accumulation and its impact on physiological and biochemical parameters of *Pisum sativum* seedlings, (ii) whether NO alleviates AgNps toxicity in pea seedlings, and (iii) mechanisms by which NO alleviates AgNps toxicity.

2. Material and methods

2.1. Collection and preparation of plant extract

The healthy leaves of *Aloe vera* plants were collected from the green house of Roxburgh Botanical Garden, Department of Botany, University of Allahabad, India where plants were cultivated under controlled environment. In a typical preparation, 15 g of thoroughly washed and finely cut fresh harvested young leaves of the plant was boiled in 100 ml sterile double distilled water for 10 min in a 500 ml conical flask with water condenser. Then the extract was collected by filtering it with Whatman filter paper no.1. The filtrate was stored at 4 °C and was used within a weak.

2.2. Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was procured from Merck, India Ltd., Mumbai, India. The reagent was of AR grade and was used without further purification. For the Synthesis of silver nanoparticles we have followed the procedure of Chandran et al. (2006) after slight modification. Further, AR grade silver nitrate ($AgNO_3$) was used as the source of silver ions. The reaction was performed at room temperature in double distilled water. In a typical reaction, 10 ml of the plant extract was added to 90 ml of 1 mM $AgNO_3$ aqueous solution under by stirring continuously for the reduction of Ag^+ ion. After the completion of the reaction, the AgNPs were collected and purified by repeated centrifugation at 15,000 rpm for 20 min followed by re-dispersion of the pellets in de-ionized water. Finally, the precipitates were dried under vacuum condition at 45 °C in a vacuum oven.

2.3. Characterizations of AgNPs

The biological reduction of Ag^+ to Ag^0 was recorded by UV–Visible spectra. It is an indirect method to examine the bioreduction of AgNPs from aqueous $AgNO_3$ solution. In a typical measurement, 100 μl of reaction mixture was diluted to 3 ml with double distilled water and the UV–Vis absorption spectra were recorded on Perkin Elmer Lambda 35 UV–Vis spectrophotometer using halogen and deuterium lamps as the sources of visible and UV radiations, respectively. The samples were scanned in a range of 200–800 nm wavelengths. After completion of reaction nanoparticles were collected and further characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDS).

XRD patterns were recorded by Rigaku D/max-2200 PC diffractometer, operated at 40 kV/20 mA, using $CuK\alpha_1$ radiation with wavelength of 1.54 Å in the wide angle region from 35° to 70° on 2θ scale and the phase identification was carried out with the help of standard JCPDS database. The crystallite size was calculated from measuring the full width at half maximum of the major XRD peak using Scherrer's formula. The size and morphology of prepared NPs were analyzed using a transmission electron microscope, model Technai 30 G^2 S-Twin electron microscope, operated at