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Gold nanoparticle exposure induces growth and yield enhancement in *Arabidopsis thaliana*

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HIGHLIGHTS

- Exposure of 24nm GNPs enhanced the total seed yield of Arabidopsis thaliana.
- GNP exposure improved growth and free radical scavenging activity of A. thaliana.
- miR expression showed correlation with growth of A. thaliana on GNP exposure.

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ABSTRACT

Nanotechnology has the potential to revolutionize agriculture field. Towards this effort, carbon nanotubes have recently been reported to induce growth enhancement of tobacco cells. In this study, exposure to 24 nm size gold nanoparticles (GNPs) at 10 μg/ml concentration was found to enhance the total seed yield of *Arabidopsis thaliana* by 3 times over the control. In addition, 24 nm size GNP exposure at both 10 and 80 μg/ml concentrations has significantly improved seed germination rate, vegetative growth and free radical scavenging activity. A considerable correlation was found between expression of key plant regulatory molecules, microRNAs (miRs) and seed germination, growth and antioxidant potential of *A. thaliana* on GNP exposure. This is the first report showing GNPs as a promising tool to enhance seed yield of plants.

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1. Introduction

Nanotechnology comprises synthesis of nano-sized (1–100 nm) particles/materials and their manipulation to generate materials or devices that can be used for various applications (Kumar and Yadav, 2009a; Biswas et al., 2012; Kumar et al., 2012). Nanomaterials have become an important ingredient of analytical methodologies, catalytic processes, DNA labels, biosensors, medicine, food industries/nutraceutics and agriculture (Daniel and Astruc, 2004; Nel et al., 2006; Mohanpuria et al., 2008; Sozer and Kokini, 2009). Application of nanotechnology to improve agricultural outcomes is an emerging discipline (Khodakovskaya et al., 2012). Enhanced efficiency of nano-based fertilizers, pesticides and other agricultural formulations can pave way to sustainable agriculture (Perez-de-Luque and Rubiales, 2009; Ghormade et al., 2011; Rai and Ingle, 2012). At the same time such products should be economical and environment friendly. This depends on the characteristics of NPs used for such applications. The properties of NPs are determined by their chemical composition, size, surface covering and most importantly, the dose at which they are used (Nature Nanotechnology Editorial, 2011; Iravani, 2011; Khodakovskaya et al., 2012).

The possibility of NP penetration into plant cells has already been ruled out (Gonzales-Melendi et al., 2008; Liu et al., 2009; Khodakovskaya et al., 2012). The dilemma that withstands is whether NPs have useful or harmful effects on plants. Reports demonstrating the effect of NPs, particularly carbon nanotubes on germination rate and seedling growth enhancement are present (Barrena et al., 2009; Seeger et al., 2009). In addition, studies have shown that NPs induce oxidative stress in plants (Barrena et al., 2009; Seeger et al., 2009; Khodakovskaya et al., 2012). Several other workers have mentioned the influence of NPs on RUBISCO, photosynthetic activity and antioxidant expression profile of plants (Lu et al., 2002; Lei et al., 2007, 2008). In order to decipher the role of nano-based products in agriculture, an extensive and illustrative work is required. As far as gold nanoparticles (GNPs) are concerned, reports documenting their effect on plant physiology, development and metabolism are very limited.

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Nevertheless there is an enhanced interest in the application of GNPs as efficient delivery system in plants to improve the agricultural outcome (Torney et al., 2007).

GNPs are among the most commonly synthesized and studied metallic NPs. They are being utilized for medical and staining purposes since 16th century. The wide applications of GNPs have initiated a huge research for their synthesis using various chemical, physical and biological routes (Daniel and Astruc, 2004; Sengul et al., 2008; Kumar and Yadav, 2009a; Lu et al., 2009). Since biological synthesis has been found to be environment friendly, several groups are increasingly synthesizing GNPs using plant extracts or microbes (Shankar et al., 2004; Kumar and Yadav, 2009a; Iravani, 2011). Biologically synthesized GNPs will be environment friendly and cost effective for various agricultural applications. To evaluate this idea, we carried out exposure studies of GNPs synthesized by *Syzygium cumini* leaf extract on the model plant *Arabidopsis thaliana*.

We exposed *A. thaliana* seeds to two different concentrations of GNPs (10 and 80 μg/ml) and studied the germination pattern, activity of antioxidant enzymes and expression pattern of microRNAs (miRs). miRs have emerged as the major regulatory molecule of plants and animals (Szymanski et al., 2003). In plants, miRs are involved in many aspects of plant physiology, including responses to abiotic stresses. The expression pattern of miRs has been observed to be modulated during stress conditions, thus influencing plant growth and development (Jones-Rhoades and Bartel, 2004; Sunkar and Zhu, 2004; Guleria et al., 2011). Keeping these facts in mind, response of GNP exposure on expression profile of miRs was also analyzed in *A. thaliana*.

2. Material and methods

2.1. NP exposure and seed germination

GNPs (24 nm) were synthesized by the same method reported previously by us (Kumar and Yadav, 2012). Plants were exposed to GNPs via germination media. The germination media was constituted by standard Murashige and Skoog (MS) salts supplemented with MS vitamins (1000×), 3% sucrose and 0.7% agar (Murashige and Skoog, 1962). The pH of media was maintained at 5.85 with 1 N NaOH. GNPs were added to media before autoclaving at a final concentration of 10 and 80 μg/ml. Germination medium without GNPs was employed as control. Seeds of *A. thaliana* were rinsed with ethanol, followed by washings in autoclaved distilled water at least three times. Seeds were surface sterilized by soaking in 0.04% mercuric chloride for 5 min and rinsed with autoclaved distilled water. Equal number of 20 sterilized seeds was placed on Petri plates containing 10 and 80 μg/ml of GNPs. The number of germinated seeds was observed till 15 days of germination and presented as percent germination rate. Relative water content and total fresh biomass of same seedlings were also evaluated. Relative water content (RWC, %) was calculated using the formula (FW − DW) / (TW − DW). Fresh weight (FW) was obtained by harvesting and weighing freshly detached rosette leaves of control as well as GNP treated plants. Turgid weight (TW) was attained by incubating cut rosettes in de-ionized water for 16 h at room temperature. TW was estimated by weighing the plant material after removing excess water by soaking with absorbent paper. Rosette dry weight (DW) was weighed after drying the turgid rosette leaves at 75 °C in a dry oven (Bouchabke et al., 2013). The data is presented as mean ± SD of mean value of three measurements.

2.2. Estimation of DPPH radical activity

DPPH free radical scavenging assay was performed as reported earlier (Joshi et al., 2011). Briefly, initial absorbance of DPPH in methanol was measured by spectrophotometer at 517 nm until the absorbance remained constant. A total of 50 μl extract was added to 1950 μl of 0.1 mM methanolic DPPH solution. The mixture was incubated at room temperature for 30 min before the change in absorbance at 517 nm was observed and percent inhibition was calculated.

2.3. Estimation of antioxidant enzyme activity

The 100 mg of fresh leaf tissue was homogenized in a pre-cooled mortar with buffer containing 2 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.5% (v/v) Triton-X 100 and 10% (w/v) polyvinyl polypyrrolidone (PVPP) in 50 mM phosphate buffer (pH 7.8) and centrifuged at 13,000 rpm for 20 min at 4 °C. This enzyme extract was used for determining the enzymes activity. SOD (EC: 1.15.1.1) activity was estimated as function of inhibition of nitroblue tetrazolium (NBT) photochemical reduction. The activity of SOD was determined as described earlier (Gill et al., 2010). APx (EC: 1.11.1.11) activity was determined following the earlier described method (Yadav et al., 2005). Total reaction mixture of 1 cm^3 contained 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA and 1.2 mM H_2O_2. The reaction was initiated by addition of enzyme extract and change in absorbance was recorded at 290 nm. The APx activity was calculated using extinction coefficient of 2.8 mM^{-1} cm^{-1}. For CAT (EC: 1.11.1.6) activity (Jannat et al., 2011), total reaction mixture of 1 cm^3 contained 50 mM potassium phosphate buffer, 500 mM H_2O_2 and enzyme extract. Change in absorbance was measured at 240 nm. The extinction coefficient of 39.4 mM^{-1} cm^{-1} was used to calculate the CAT activity. GR (EC: 1.6.4.2) enzyme activity was determined as described earlier (Kumar and Yadav, 2009b). Total reaction mixture contained 50 mM potassium–phosphate buffer (pH 7.0), 0.8 mM EDTA, 0.5 mM GSSG, 0.2 mM NADPH and enzyme extract. Change in absorbance was read at 340 nm and was used to calculate GR activity.

2.4. Small RNA isolation

Small RNA fraction was isolated from 15 day old control (no GNP exposure) as well as 10 and 80 μg/ml GNP exposed seedlings. Isolation was carried out using Qiagen miRNeasy Plant Minikit as per the manufacturer's instructions. The quality and quantity of isolated small RNA samples were measured using Nanodrop ND-1000 (Nanodrop Technologies, USA).

2.5. Stem-loop reverse transcription PCR

Stem-loop reverse transcription (SL-RT) primers were designed manually for miR408, miR399, miR398, miR397, miR395, miR319, miR169, miR167, miR164, and miR414 as described earlier (Varkonyi-Gasic et al., 2007; Guleria and Yadav, 2011). The 50 ng of small RNA fractions was used to synthesize miR-specific cDNA by stem-loop reverse transcription. The 0.5 μl of 10 mM dNTP mix was added to RNA, incubated for 5 min at 65 °C and then kept on ice for 2 min. To the above mix, 2 μl of 5× First Strand Buffer, 1 μl of 0.1 M DTT and 0.5 μl of Superscript III RT (100 units) were added. Master mix of the above recipe was distributed equally in 10 tubes and 1 μl of each of miRNA specific stem-loop RT primers was added separately. The reaction was conducted as follows: 16 °C, 30 min for 1 cycle; 30 °C, 30 s; 42 °C, 30 s; and 50 °C, 1 s for 60 cycles followed by 85 °C, 5 min.

2.6. microRNA expression analysis

Expression evaluation of miRs was performed by reverse transcription-polymerase chain reaction (RT-PCR) with the above synthesized cDNAs. For RT-PCR amplification, miR-specific forward primer and a common reverse primer were used (Varkonyi-Gasic et al., 2007; Guleria and Yadav, 2011). The 20 μl reaction mix comprised of 1 μl miR-specific cDNA, 0.4 μl forward primer (10 μM), 0.4 μl reverse primer (10 μM), 0.4 μl of 10 mM dNTP mix, 2 μl of 10× PCR Buffer and 0.4 μl of Taq polymerase. The reaction was

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