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A nanosized Ag–silica hybrid complex prepared by γ-irradiation activates the defense response in Arabidopsis

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ABSTRACT

Silver nanoparticles have antimicrobial activity against many pathogenic microbes. Here, the preparation of a nanosized Ag–silica hybrid complex (NSS) prepared by γ-irradiation is described. The effects of both NSS and reduced Ag nanoparticles (Ag^0) on the growth of the model plant *Arabidopsis thaliana* were tested. The application of 1–10 ppm NSS complex improved Arabidopsis growth in soil, whereas 100 ppm NSS resulted in weakly curled leaves. In addition, supplementation of Murashige and Skoog (MS) growth medium with 1 ppm NSS promoted the root growth of Arabidopsis seedlings, but root growth was inhibited by supplementation with 10 ppm NSS. To investigate whether the NSS complex could induce plant defense responses, the expression of pathogenesis-related (*PR*) genes that are implicated in systemic acquired resistance (SAR) in Arabidopsis plants was examined. *PR1*, *PR2* and *PR5* were significantly up-regulated by each application of 10 ppm NSS complex or Ag^0 to the rosette leaves. Furthermore, pretreatment with the NSS complex induced more pathogen resistance to the virulent pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) compared to water treatment in Arabidopsis plants.

1. Introduction

Nanotechnology is the rapidly growing science of producing and utilizing particles that measure in the 1–100 nanometer (nm) range. It has the potential to create numerous new materials and devices with a vast range of applications, in areas such as medicine, agriculture, electronics, biomaterials and energy production. Nanoparticles of metals such as copper (Cu), silver (Ag) and gold (Au) have been the focus of great interest due to their unique optical properties (Kreibig and Vollmer, 1995). In particular, silver nanoparticles have been shown to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities. Different approaches have been employed to prepare a range of nanomaterials, including physical mixing (Akelah et al., 1995), the sol–gel technique (Jang et al., 2000), *in-situ* chemical polymerization (Ray and Biswas, 2000), emulsion technology (He, 2005), the sono-chemical process (Kumar et al., 2000), spray pyrolysis (Kim et al., 2008a), the unsymmetrical square wave current method (Zhou et al., 2006), the photo-redox chemical process (Khanna et al., 2005), the electrochemical method (Kim et al., 2008b) and the γ-irradiation technique (Henglein, 1993; Kim et al., 2010; Choi et al., 2003). The γ-irradiation technique is a simple method and has been widely used to generate nanoscale metals (Henglein et al., 1995) and nanocomposites (Yin et al., 1998) at room temperature and normal pressure. Similarly, Park et al. (2006) developed a new composition of nanosized silica–silver using γ-irradiation to control various plant diseases. It consisted of nano silver combined with silica molecules and a water-soluble polymer and was prepared by exposing a solution of silver salt, silicate and water-soluble polymer to radioactive γ-rays.

The successful use of nanosized silver particles in diverse medical applications as antifungal and antibacterial agents (Panacek et al., 2009; Singh et al., 2008) has led to their use in controlling phytopathogens. Nanosized silver particles with broad spectrum antimicrobial activity were shown to reduce the severity of various plant diseases caused by spore-producing fungal pathogens (Jo et al., 2009; Kim et al., 2009; Min et al., 2009; Park et al., 2006). Park et al. (2006) also showed that the nanosized

Abbreviations: NSS, nanosized Ag–silica hybrid complex; Ag^0, reduced Ag nanoparticles; PR, pathogenesis-related; SAR, systemic acquired resistance; Pst, *Pseudomonas syringae* pv. *tomato* DC3000

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silica–silver particles prepared by γ-irradiation exhibit a wide range of antimicrobial activity by controlling spores and hyphae. Recently, Min et al. (2009) suggested the possibility of using silver nanoparticles as an alternative to pesticides for the control of scleotium-forming phytopathogenic fungi. The application of silver nanoparticles severely damaged the fungal hyphae by separating the layers of the hyphal wall and collapsing the hyphae. However, so far, there are no reports of the effects of nanoparticles on plants at the molecular level. SAR is an inducible defense mechanism and plays a vital role in defending plants from various types of pathogen attack (Durner et al., 1997). SAR has been well documented in tobacco and Arabidopsis plants, and a set of *PR* genes such as *PR1*, *PR2* and *PR5* has been identified as SAR marker genes (Delaney et al., 1994; Gaffney et al., 1993).

Here, we describe the preparation of nanosized Ag–silica hybrid complex using the γ-irradiation technique for applications in agriculture. We report the effects of two types of nanosized silver particles on plant growth and induced systemic resistance of the model plant *Arabidopsis thaliana*.

2. Materials and methods

2.1. Chemicals

Silver nitrate ($AgNO_3$, 99.8%) and sodium silicate (Na_2SiO_3) solutions were prepared using analytical reagent grade chemicals (Samchun Chemical). Isopropanol (IPA, 99%) and polyvinylpyrrolidone (PVP, 99.9%) were purchased from Merck and Acros, respectively. All solutions were prepared using deionized distilled water (DDW) with a resistance of 18.2 MΩ cm^{-1}.

2.2. Preparation of nanosized Ag–silica hybrid complex

Silver ions (Ag^+) are reduced to the free silver atom (Ag^0) by ionizing radiation in aqueous solution (Janata et al., 1994). In this paper, we prepared both the reduced silver nanoparticles (Ag^0) and the nanosized silica-hybrid silver (NSS) complex. The Ag^0 nanoparticles were prepared in the absence of silicate molecule as described below. The NSS complex was prepared as follows. $AgNO_3$ (1.0 g), Na_2SiO_3 (1.0 g), PVP (1.0 g) and IPA (12 ml) were dissolved in a final volume of 200 ml DDW. Oxygen was removed from the solution by bubbling with pure N_2 gas for 30 min and the resultant solution was irradiated with 30 kGy for 3 h using a ^{60}Co γ-irradiator (150 TBq capacity; ACEL, Canada) at the Korea Atomic Energy Research Institute (Park et al., 2006, 2007; Marignier et al., 1985). The morphology of silver nanoparticles was determined using images acquired with an SU-70 Field Emission-Scanning Electron Microscope (FE-SEM) with an Energy Dispersive X-ray (EDX) system (HITACHI, Japan).

2.3. Plant treatments, RNA extraction and RT-PCR analysis

The model plant *Arabidopsis thaliana* (ecotype Columbia) was used in this work. Plants were grown to maturity under long-day conditions at 22 °C. For the leaf morphology assay, the rosette leaves still attached of four-week-old plants grown in soil were evenly sprayed with various concentrations of NSS or Ag^0 (1, 10, 50 and 100 ppm). For the root growth experiment, Arabidopsis seeds were germinated on vertical MS medium plates supplemented with NSS or Ag^0, both at concentrations of 1 ppm and also at 10 ppm, and kept under long-day conditions at 22 °C until 1 week after germination. The primary root length of seedlings was measured at 3 and 5 days after germination. For the expression analysis of SAR marker genes by reverse transcriptase-polymerase chain reaction (RT-PCR), 4-week-old plants were sprayed with 10 ppm NSS or Ag^0 and water as a control. Rosette leaves were collected at the indicated time points after treatment. Total RNA was extracted from the samples using the RNA Extraction Kit (iNtRON). First-strand cDNAs were synthesized from 2.5 μg of total RNA using a RevertAid™ First-strand cDNA Synthesis Kit (Fermentas). RT-PCR was performed in 50 μl reactions containing 2 μl of 1:10 diluted cDNA samples and gene-specific primers using Ex-Taq DNA polymerase (PanVera) under the following conditions: an initial denaturation step at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s, polymerization at 72 °C for 1 min and a final extension at 72 °C for 10 min. Control RT-PCR was performed using a primer pair specific to the *ACTIN2* (At3g18780) gene under the same conditions. From each reaction, 10 μl of RT-PCR product was analyzed on a 1.0% agarose gel. The gene-specific primers used for RT-PCR analysis were as follows: *PR1* (forward: 5′-ATCGTCTTTGTAGCTCTTGTAGGTG-3′, reverse:

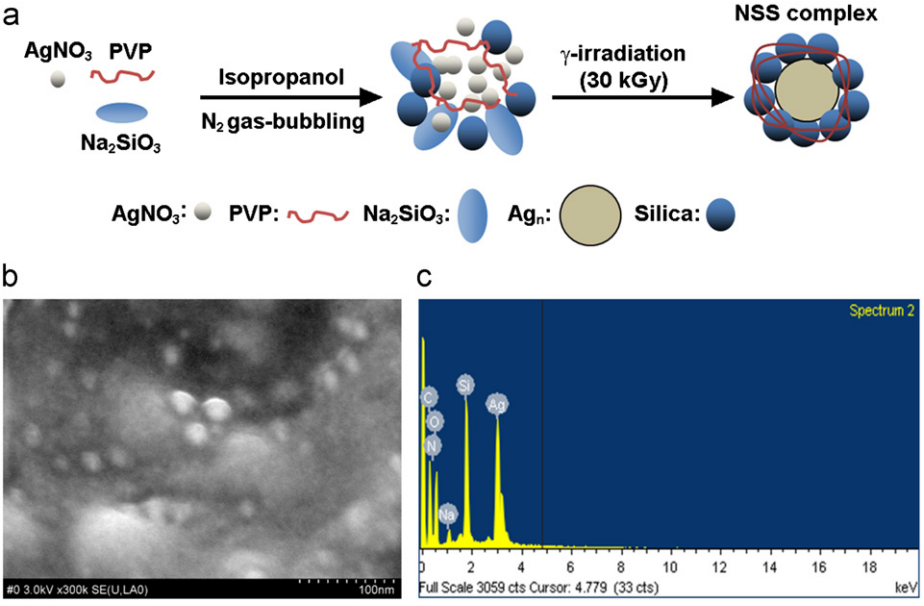

Fig. 1. Preparation of the nanosized Ag–silica hybrid complex by γ-irradiation (a) and the corresponding FE-SEM images (b) and EDX spectrum (c).