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Genotoxicity effects of silver nanoparticles on wheat (*Triticum aestivum* L.) root tip cells

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ABSTRACT

The distribution and use of nanoparticles have rapidly increased over recent years, but the available knowledge regarding their mode of action, ecological tolerance and biodegradability remains insufficient. Wheat (*Triticum aestivum* L.) is the most important crop worldwide. In the current study, the effects of silver nanoparticles (AgNPs) obtained from two different sources, namely, green and chemical syntheses, on chromosomal aberrations and cell division were investigated. Wheat root tips were treated with four different AgNP concentrations (10, 20, 40 and 50 ppm) for three different exposure durations (8, 16 and 24 h), and the different concentrations of the nanoparticles were added to the tested grains until the root lengths reached 1.5–2 cm. For each concentration, the mitotic indexes (%) were obtained from an analysis of ~ 2000 cells. The treated root-tip cells exhibited various types of chromosomal aberrations, such as incorrect orientation at metaphase, chromosomal breakage, metaphasic plate distortion, spindle dysfunction, stickiness, aberrant movement at metaphase, fragmentation, scattering, unequal separation, scattering, chromosomal gaps, multipolar anaphase, erosion, and distributed and lagging chromosomes. These results demonstrate that the root tip cells of wheat can readily internalize the AgNPs and that the internalized AgNPs can interfere with the cells' normal function.

1. Introduction

Nanoparticles (NPs) are zero-dimensional, 1- and 100-nm crystallites that behave as a single unit regarding their transport and properties. Nanoparticles have a wide range of commercial applications; in fact, silver NPs (AgNPs) are used to produce more than 250 products worldwide (Jiang et al., 2012). NPs can be derived from both natural and anthropogenic sources (Biswas and Wu, 2005; Nowack and Bucheli, 2007; Tervonen et al., 2009) and exert cytotoxic and genotoxic effects in plants, including lipid peroxidation, decreases in the mitotic index (MI), and enhancement of the micronuclei and chromosomal aberration indexes (Kumari et al., 2011).

Different NPs have distinct effects on root growth, which also vary among plant species. Moreover, certain NPs, such as CuO NPs, can cause extensive DNA damage in some agricultural and grassland plants (Rodriguez et al., 2011). Similarly, Manosij et al. (2016) observed a loss of membrane integrity, increased chromosome aberrations, micronucleus formation, DNA strand breaks, and cell cycle arrest at the G2/M checkpoint in *Allium cepa* following exposure to ZnO nanoparticles (diameter = ~ 85 nm). In *Vicia faba* and *Nicotiana tabacum*, NPs increased intracellular ROS production, lipid peroxidation, and antioxidant enzyme activities (Manosij et al., 2016). The NPs were internalized, leading to notable alterations in cell morphology.

A study of the interaction between AgNPs and *V. faba* revealed an NP size- and exposure-dependent effect on the MI and chromosomal aberrations (Abdel-Azeem and Elsayed, 2013). Additionally, decreases in the nanoparticle size were associated with decreases in the MI and root growth values with increasing treatment time (h) and increases in the numbers of aberrant cells. Several changes in mitosis, such as disturbed chromosomes at metaphase and anaphase, laggards, fragments, bridges, chromosome stickiness and micronuclei (Mn), were observed. Similarly, Yin et al. (2012) reported that AgNPs have different toxicities based on their size and shape, which can affect cell wall penetration (Morones et al., 2005; Carlson et al., 2008).

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Several studies have revealed the ecotoxicity of AgNPs. In fact, AgNPs reduce cell growth, photosynthesis, and chlorophyll production in a marine diatom (*Thalassiosira weissflogii*) and in fresh water algae (*Chlamydomonas reinhardtii*), and these toxic effects might be due to the release of dissolved silver upon dissolution of the AgNPs (Navarro et al., 2008a, 2008b; Miao et al., 2009). Kumari et al. (2011) reported that AgNPs cause impairments in various stages of cell division and cell disintegration in the root tips of onion.

Moreover, Patlolla et al. (2012) demonstrated that exposure to AgNPs significantly increases the numbers of chromosomal aberrations and micronuclei and decreases the MI compared with the control, and Mazumdar (2014) recently revealed that once AgNPs enter cells of *Vigna radiata* and *Brassica campestris*, it can cause damage to vacuoles and cell wall integrity and likely affect other organelles. Additionally, the observed retardation of growth during the seedling stage was due to considerable absorption of by the root cells. With an increase in the concentration of AgNPs (up to 60 mg/ml), those particles penetrated the cell wall and damaged the cell morphology and its structural features of *Oryza sativa* plants (Mirzajani et al., 2013).

Sequentially, the present investigation explored the effects of AgNPs on the MI and chromosomal aberrations in wheat root tips.

2. Materials and methods

Root tips of wheat (*Triticum aestivum* L.) (2n = 42) were used as the study material. Ten healthy grains were grown in a Petri dish (7.5 cm) at room temperature, and distilled water was added to each cultivar until germination (two days). Three different exposure times (8, 16 and 24 h) and four AgNP concentrations (10, 20, 40 and 50 ppm in a total volume of 20 ml of dH_2O) were tested. The nanoparticles were added to the grains until the root length reached 1.5–2 cm, and for each NP solution, the MIs (%) were measured through analyses of ~ 1093–2000 cells. These steps are summarized in Fig. 1.

To detect the different stages of mitosis division we should use the root tip or meristems in length from 1 to 1.5 cm so the treatments were done after the first day when grain start in germination using three different expose time 8, 16 and 24 h. Because if we wait for other days the roots will increase in length and we will loss these stages (Gömürgen, 2005; Babu et al., 2007; Asita and Mokhobo, 2013; Elena et al., 2013; Abdel-Azeem and Elsayed, 2013; Abou-Zeid and Moustafa, 2014; Raskar and Laware, 2014; Ghosha et al., 2016).

2.1. Green synthesis of AgNPs

The AgNPs were synthesized according to the method described by Abdel-Megeed et al. (2015) with a slight modification. Briefly, 10 ml of algae extraction buffer was added to 90 ml of 0.001 M $AgNO_3$ at room temperature, and the reaction mixture was incubated for 1 h in the dark. A gradual change in the colour of the mixture from greenish yellow ($AgNO_3$ solution + algae extraction) to dark brown colour was observed, indicating the formation of AgNPs (Sivalingam et al., 2012).

Species of the marine Rhodophyta (*Corallina elongata* Ellis et Solander) were hand-picked in September 2016 from Rocky Bay of Abu Qir (at a longitude of 30°03′ to 30°22′E and a latitude of 31°16′ to 31°28′N), Alexandria, Egypt. The algal samples were transported to the laboratory in an ice box and washed numerous times with filtered sea water to remove epiphytes, sand particles and surface salts.

The cleaned algae were dried under shade for 48 h until complete moisture removal was achieved and the samples reached a constant weight. The dried samples were finely ground in a mortar and stored in a desiccator until further use. Five grams of the dried finely powdered algal samples were dispersed in 100 ml of distilled water in an Erlenmeyer flask by magnetic stirring and heating to 100 °C for 20 min. The extract was filtered through mesh and then a 0.2-µm Millipore filter and stored at − 20 °C for further use.

2.2. Chemical synthesis of AgNPs

AgNPs were chemically synthesized according to the method developed by Aslani et al. (2004) with slight modifications. Sodium hydroxide solution (4.0 M, 10 ml) was added to a solution of $AgNO_3$ (2 mM) in $EtOH/H_2O$ solvent (25 ml). To investigate the role of surfactants on the size and morphology of NPs, 0.5 g of polyethylene glycol (PEG) was used in the reactions under optimized conditions. The

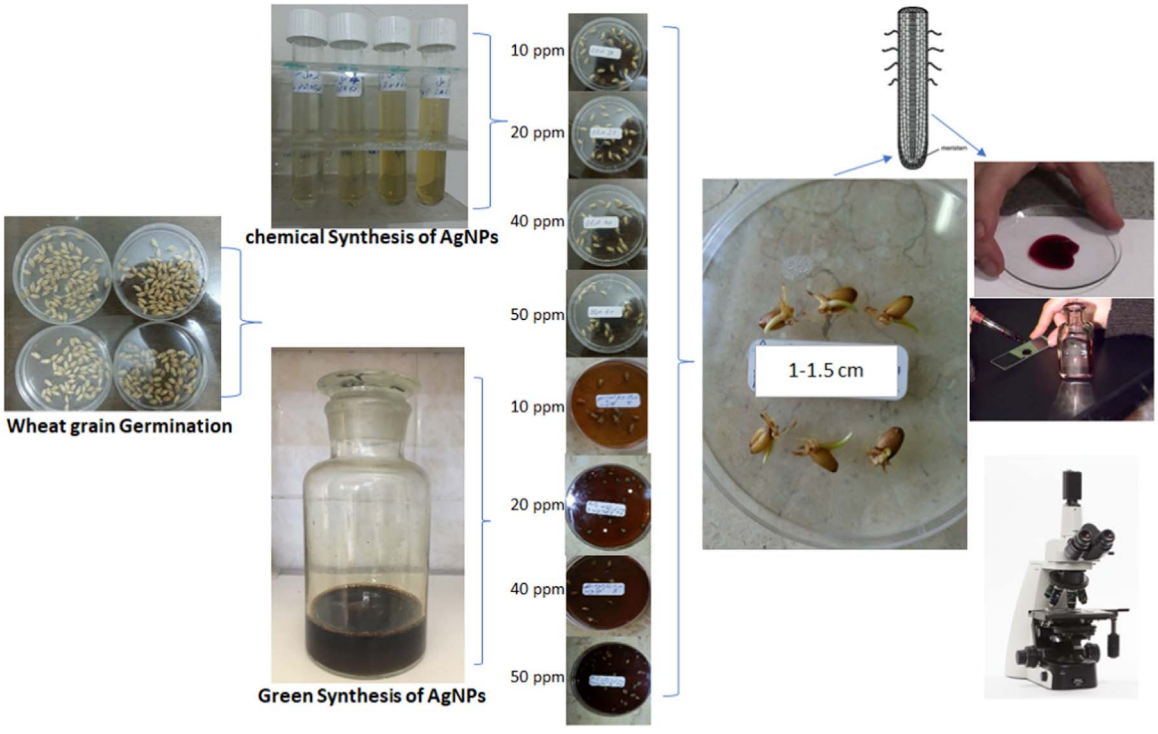

Fig. 1. Steps of the experiment for AgNPs synthesis and wheat grain exposure to its concentrations.