

## Analysis of Plant Responses to Titanium Dioxide (TiO<sub>2</sub>) Nanoparticles

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Recently, the amount of nanoparticles in consumer products has dramatically increased. Because of their broad scale use, these nanomaterials can be expected to be present in the environment, raising concerns about their effects on the lives of plants and animals. If these nanomaterials reach the environment, plants could potentially interact with them and take-up the particles into their system. Titanium dioxide (TiO<sub>2</sub>), a nanomaterial, is used in the production of cosmetics, and has been shown to have UV light shielding capabilities [2]. The effect of TiO<sub>2</sub> nanoparticles on metabolic processes has been studied and was found to affect processes such as hormone metabolism [1]. However, the structural effects of TiO<sub>2</sub> nanoparticles are still unclear. This study focuses on observing the effects of 5-15 nm TiO<sub>2</sub> nanoparticles on the physiology and structure of cells and organelles of two plant species [jalapeno (*Capsicum annuum*) and corn (*Zea mays*)] using light and electron microscopy techniques. Plant responses were a result of nanoparticle exposure through the root systems.

The plants were grown from seeds in a growth chamber with controlled light, humidity and temperature. Six plants of the same height from each species were collected, their roots washed thoroughly, and transferred to bowls containing a soil-free liquid culture medium (Hoagland solution) (Fig. 10). On the seventh day, the solution in three of the four bowls was replaced with three concentrations of TiO<sub>2</sub> (5-15nm) nanoparticles (300 mg/L, 600 mg/L, and 1000 mg/L) dissolved in water. The final dish was filled with water and served as the control. The plants were maintained for at least seven days for the uptake of TiO<sub>2</sub>. Root and leaf samples from each group were collected and washed for study using light and electron microscopy. Root samples collected for SEM were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in series of ethanol, critically dried in a critical point dryer, mounted on aluminum stubs, gold sputter coated, and observed under the Tescan Vega3 SEM (includes a Thermo-Noran EDS detector) for the presence of titanium. Samples for TEM and light microscope (LM) analysis were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in series of ethanol, incubated in acetone, and embedded in EMBed 812. Sectioned blocks were used for LM and TEM analyses. The root mass of corn and jalapeno plants exposed to high levels of TiO<sub>2</sub> was lower than that of the control indicating inhibition of root growth (Fig.9). Results from SEM analyses exhibited structural damage of root epidermal cells in corn and jalapeno plants exposed to 1000 mg/L TiO<sub>2</sub> (Fig.2). EDS analyses indicated presence of titanium particles on the surface of both corn and jalapeno plant roots exposed to 1000 mg/L TiO<sub>2</sub> (data not shown). The control groups did not show any signs of structural damage (Fig. 1). Light microscopy revealed possible vacuolation in the corn root cells exposed to large amounts of TiO<sub>2</sub> (Fig. 4). This was most evident when comparing the corn control to the 1000 mg/L TiO<sub>2</sub> sample (Fig.3). TEM analysis uncovered changes in the roots of both jalapeno and corn 1000 mg/L TiO<sub>2</sub> groups. In comparison to the control, the 1000 mg/L TiO<sub>2</sub> corn root cells became highly vacuolated with no apparent presence of TiO<sub>2</sub> particles inside the cells (Fig.5 & 6). Therefore corn, a monocot plant, may not take-up titanium nanoparticles. This phenomenon is yet to be understood. TEM analysis of the jalapeno plants treated with 1000 mg/L TiO<sub>2</sub> revealed clusters of particles (possibly TiO<sub>2</sub>) within the root cells, but no vacuolization (Fig.7 & 8). While the root surface structural damage seen in SEM images appears to be the same in both species, TEM analysis demonstrated that TiO<sub>2</sub> might have varying effects on the cells of corn and jalapeno plants. Further experiments are underway to evaluate the chlorophyll levels in the leaves of each species for each experimental group.

References:

1. Laxminath, Tumburu, et al. "Phenotypic and Genomic Responses to Titanium Dioxide And Cerium Oxide Nanoparticles In Arabidopsis germinants." *Environmental Toxicology and Chemistry* 34.1 (2015): 70-83.
2. Li, Xiaozho, et al. "Layer-by-layer assembled TiO<sub>2</sub> films with high ultraviolet light-shielding property." *Thin Solid Films* 571.1 (2014): 127-133.

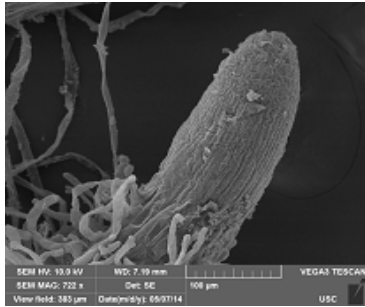


Fig.1: Control jalapeno root tip with no visible structural damage (SEM)



Fig.2: 1000 mg/L TiO<sub>2</sub> jalapeno root tip with visible structural damage (SEM)

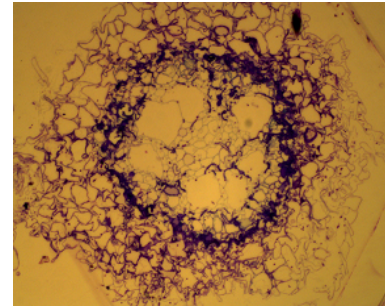


Fig.3: Corn control root cross section without structural damage (LM)

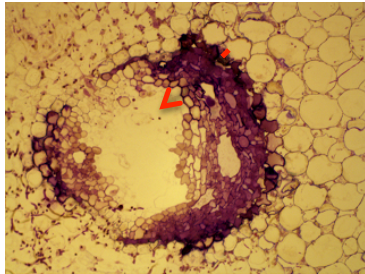


Fig.4: 1000 mg/L TiO<sub>2</sub> corn root cross-section with possible vacuolization (LM)

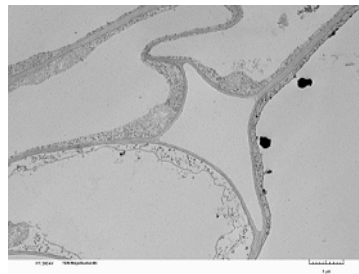


Fig.5: Corn control root cells showing no vacuolization (TEM)

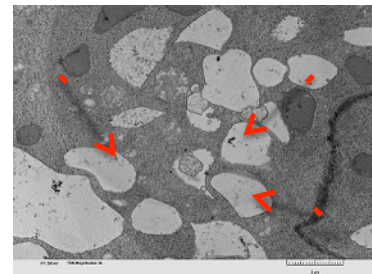


Fig.6: 1000 mg/L TiO<sub>2</sub> corn root cells with visible vacuolization (TEM)



Fig.7: Jalapeno control root cells with no visible particle clusters (TEM)

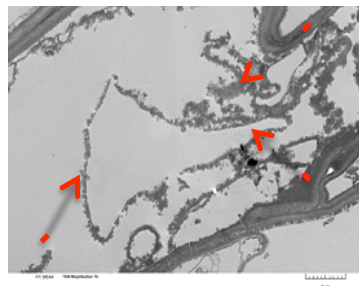


Fig.8: 1000 mg/L TiO<sub>2</sub> jalapeno root cells with visible particle clusters within the cells (TEM)

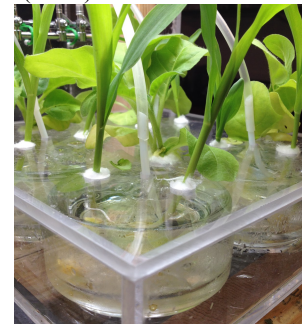


Fig.10: Method for exposure of plants to TiO<sub>2</sub>



Fig.9: Dry root mass in grams of corn and jalapeno control and experimental groups