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Structural Analysis of Plants Exposed to Titanium Dioxide (TiO_2) Nanoparticles

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Nano-sized material production and application have advanced rapidly in recent years and they have been used in a number of consumer products. Due to their wide scale use, various nanomaterials are expected to reach the environment and accumulate over time. This will likely create a new class of environmental pollutants and therefore, the assessment of their potential impact on plant and animal life is essential. Plants are likely to come in contact with various types of nanoparticles in the environment and this exposure may result in uptake and accumulation in various plant tissue types. A handful of recent studies have demonstrated the ability of nanoparticles to interact with plants [*1*]. However, the results tend to vary to a great extent and various plant species may respond differently to nanoparticles in the environment. Titanium dioxide (TiO_2) is a commonly used nanomaterial in many applications including sunscreens, paints and photocatalysis. TiO_2 in suspension has exhibited leaf growth inhibition in maize [*2*]. However, the environmental impacts of TiO_2 nanoparticles in plants still remain unclear. We describe here the effects of anatase TiO_2 nanoparticles (5-15nm) on three plant species, tobacco (*Nicotiana tabacum*), mustard (*Brassica juncea*) and jalapeno (*Capsicum annuum*) by electron microscopy techniques. In this study we primarily investigated the uptake of TiO_2 particles through the plant root system and subsequent response.

Plants were grown from seeds in potting soil mix under controlled temperature, humidity, and light condition, then moved to the hydroponic Hoagland's Solution (pH 6.5) and maintained for 3-4 days to acclimate in the soil free environment. After acclimation, the solutions were replaced with TiO_2 nanoparticles dissolved in water at the concentrations of 0 (control), 75, 150, and 300 mg/L and the plants were maintained for 7-8 days (fig. 6). At the end of 7^{th} or 8^{th} day of TiO_2 exposure, plant root specimens from control and experimental groups were washed vigorously, fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in ethanol, critically dried, gold sputter coated, and viewed and analyzed under a Tescan Vega-3 SBU SEM outfitted with a Thermo Scientific EDS detector. SEM and EDS analyses of roots of all three plant species treated with TiO_2 confirmed the presence of titanium on the surface of roots even after extensive washing of roots prior to fixation (fig 3, 4, 5). Structural deformities and damages of root epidermal cells in mustard plants were observed in the 300mg/L TiO_2 groups, indicating a negative impact of the nanoparticles on the plant (fig. 2). The mustard plants exposed to 75 & 150 mg/L TiO_2 showed enhanced growth indicating a possible growth stimulation triggered by TiO_2. On the contrary, tobacco and jalapeno roots exposed to TiO_2 did not show any morphological deformities indicating possible exclusion of TiO_2 by its root system or hyper-accumulation of TiO_2. The control group of roots with no TiO_2 did not exhibit any sort of structural damage (fig. 1). Therefore, mustard might be more susceptible to TiO_2 exposure than tobacco or jalapeno. Further experiments are in progress to evaluate the effect of TiO_2 at subcellular level.

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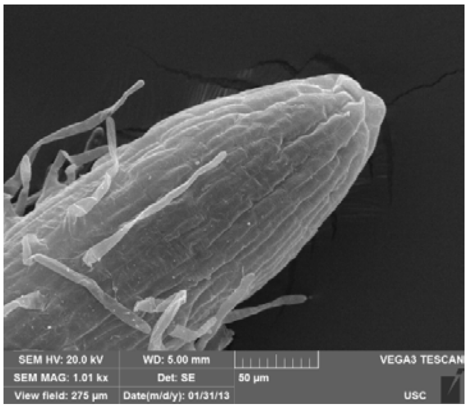

Fig. 1 Control mustard root tip showing no structural damage

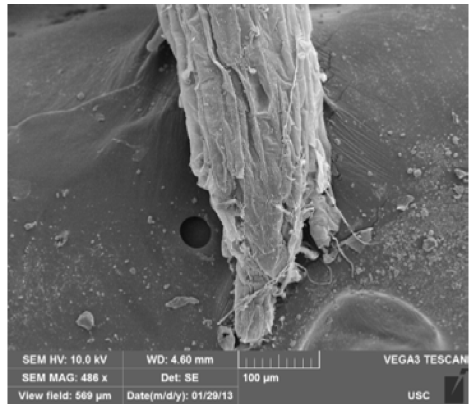

Fig. 2 Mustard root tip (300mg/L TiO_2) showing structural damage

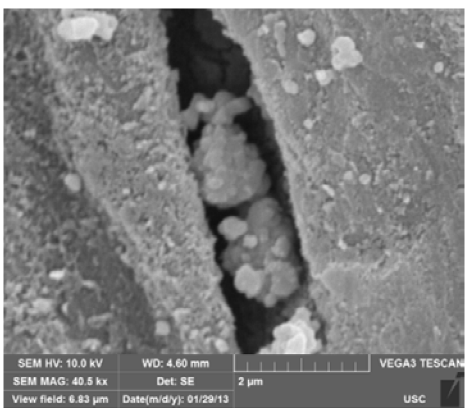

Fig. 3 Root tip epidermis showing TiO_2 clusters

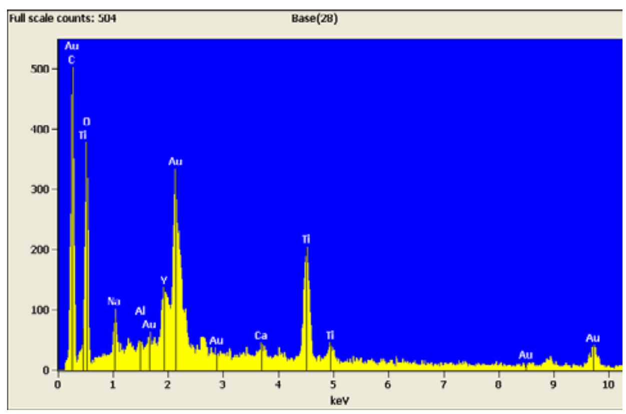

Fig. 4 EDS spectrum showing presence of TiO_2 on root epidermis of all treatment groups

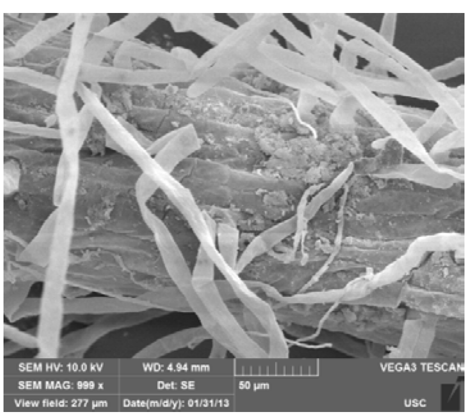

Fig. 5 Mid-section of TiO_2 exposed roots showing TiO_2 particle clusters on the surface, but no structural damage in tobacco and jalapeno

Fig 6 Experimental set up for TiO_2 exposure to tobacco, mustard, and jalapeno plants

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