

Mitigation of TiO₂ Stress and Characterization of a Brassinosteroid Insensitive-1 Gene in Aquatic Bioindicator Species *Lemna minor*

Ye, Tong (School: Half Hollow Hills High School East)

Makani, Dylan (School: North Shore Hebrew Academy High School)

Balestrieri, Jacqueline (School: Saint Anthony's High School)

Titanium dioxide nanoparticles (TiO₂ NPs) are an abundant heavy metal stressor frequently discharged as industrial effluent. There has been little research on the effect of TiO₂ NPs on aquatic organisms and their respective ecosystems. The brassinosteroid, 24-Epibrassinolide (24-EBL), regulates plant growth and stress through a Brassinosteroid Insensitive-1 (BRI1) pathway as shown in past literature. This study investigated the effects of TiO₂ NPs on *Lemna minor*, an understudied, aquatic bioindicator that is an important component of biofuel. Through physiological, molecular, and in silico analysis of *L. minor*, a previously undiscovered Brassinosteroid Insensitive-1 (BRI1) pathway was identified and found to effectively mitigate stress from TiO₂ NPs. Exposure to TiO₂ NPs decreased growth and concentrations of photosynthetic pigments in *L. minor*. However, after a pretreatment of 24-EBL, plants displayed frond area, number, and root lengths similar to control, indicating that 24-EBL attenuates the harmful effects of TiO₂ NPs in *L. minor*. Our discovery could lead to a better understanding of how effective *L. minor* is as a bioindicator, since it seems to possess a stress alleviating pathway that other organisms may not have. Additionally, a putative BRI1 sequence was successfully extracted from *L. minor* and it was concluded that pretreating *L. minor* with 24-EBL solution prior to TiO₂ NP exposure resulted in stress reduction. Upon investigation using RT-PCR, the extracted fragment appeared to be upregulated when exposed to TiO₂ NP. Future studies can examine how *L. minor* and other aquatic organisms expressing the BRI1 genetic pathway can contribute to the protection of global water systems and the sustainability of aquatic communities.