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RESEARCH ARTICLE

INFLUENCE OF TITANIUM DIOXIDE NANOPARTICLES ON THE PHOTOSYNTHETIC AND BIOCHEMICAL PROCESSES IN ORYZA SATIVA

Maria Vera J. Da Costa and *Prabhat K. Sharma

Department of Botany, Goa University, Goa

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ABSTRACT

The phytotoxicity of titanium dioxide nanoparticles (TiO_2 NPs) is a rapidly expanding area of research with a dearth of information regarding its toxicity in plants. We assessed the TiO_2 NP (<25 nm) effect on growth and photosynthesis in *Oryza sativa* var. Jyoti grown in a hydroponic system. Our results show that TiO_2 NPs do not affect germination, however, an overall decrease in seedling growth (root, shoot length and biomass) was observed at high concentration. TiO_2 NP exposure led to greater accumulation of Ti in roots than in shoots, decreasing CO_2 fixation, transpiration rate and stomatal conductance at 1000 ppm with insignificant effect on photosynthetic pigments, quantum efficiency of PSII (Fv/Fm ratio) and photochemical quenching. High treatment caused oxidative and osmotic stress evidenced by increased MDA and proline content. This study thus indicates that Ti accumulation caused phytotoxicity to growth and photosynthesis leading to oxidative and osmotic stress in rice.

INTRODUCTION

Metal oxide nanoparticles (NPs) are materials with size range of 1-100 nm (Klaine *et al*., 2008). Titanium dioxide (TiO_2) NPs are one of the most commonly found particles in the environment as a result of their large scale production and application for commercial purposes such as cosmetics, sunscreens (Mu *et al*., 2010), semiconductors, pharmaceuticals, energy storage, catalysts, coatings and paints (Kaegi *et al*., 2008), due to its UV absorption efficiency and transparency to visible light (Franklin *et al*., 2007). It is also used in the production of textiles (Yuranova *et al*., 2007) and for solar-driven self-cleaning coatings (Cai *et al*., 2006). These usages would inevitably lead to increased NP entry into the environment. As far as concentration of NPs in soil or aquatic system is concerned, no direct published data is available (Navarro *et al*., 2008). Using fate models, Mueller *et al*. (2008), estimated the concentration of TiO_2 NP to be 120 $\mu g/kg^3$ of agricultural land per year as a result of contaminated sewage sludge. Boxall *et al*. (2007) used simple box models to estimate the amount of Ti in the environment and reported that the expected amounts of Ti and other common NPs (Ce, Zn and Ag) were found to be in the range of 1 to 10 µg/L in natural waters, with total NP concentration scaling up to 100 µg/L approximately in the aquatic system currently which may increase many fold in the near future.

Release of TiO_2 NP in the environment is ameliorating at a very rapid rate contaminating soil and aquatic ecosystems (Farre *et al*., 2009; Tourinho *et al*., 2012). TiO_2 NPs are likely to persist and accumulate in the soil and water due to low dissolution (Baun *et al*., 2008) forming stable aggregates (French *et al*., 2009). Agglomeration and aggregation, in the environment are influenced by NP surface characteristics and particle size. These are negatively correlated to soil characteristics such as dissolved organic matter, and positively correlated to pH and ionic strength (Fang *et al*., 2009).

The mechanism of NP uptake by plant roots is not clearly understood. It is reported that depending on the size nanoparticles, NPs may enter the plant cells through carrier protein, aquaporin, ion channels, fluid phase endocytosis, plasmodesmata transport or entry facilitated through natural organic matter or root exudates and formation of new pores (Rico *et al*., 2011). Once inside the cell, NPs can bind to the organelles or protein (Rosenbluh *et al*., 2011) or form aggregates (Guzman *et al*., 2006). Interaction of NPs in plants not only depends upon the surface characteristics, particle size, temperature, pH and ionic strength (Maynard *et al*., 2006; Wiesner *et al*., 2006) but also on the type of interactions with lipids and proteins such as electrostatic, hydrogen bonding, and hydrophobic interactions (Hug and Sulzberger, 1994; Ojamae *et al*., 2006).

Reports on uptake of TiO_2 NP in plants are limited, especially in food crops (Rico *et al*., 2011). Uptake, translocation and cell distribution of ultra small TiO_2 NPs (<5 nm) in *Arabidopsis thaliana* was reported to be either facilitated or inhibited by the pectin hydrogel capsule which was released by the roots (Kurepa *et al*., 2010). Increased accumulation or inactivation of toxic heavy metals by the rhizosphere was also reported to be dependent on plant species (Watanabe *et al*., 2008). TiO_2

Corresponding author:* **Prabhat K. Sharma
Department of Botany, Goa University, Goa

NPs toxicity caused reduction in root cell diameter, hydraulic conductivity, transpiration and leaf growth in maize (Asli and Neumann, 2009) and produced reactive oxygen species (ROS) within organisms due to its photocatalytic activity upon UV exposure (Kahru *et al*., 2008). TiO_2 NPs are reported to be beneficial to some organisms. For instance, in spinach, TiO_2 NPs increased chlorophyll synthesis, photosynthesis and plant dry weight (Zheng *et al*., 2005; Hong *et al*., 2005).

The multidisciplinary application of nanotechnology-products results in the inevitable discharge of NPs into the environment (Navarro *et al*., 2008). Problems and challenges arise due to phytotoxicity exhibited by the NPs owing to their physicochemical surface characteristics upon interaction with the living system. The lack of knowledge and limited information on the fate and bioavailability of NPs in the environment also augment these problems (Klaine *et al*, 2008). Our study provides a better understanding of the interaction of TiO_2 NP in *Oryza sativa* var. Jyoti and suggests that light reaction of photosynthesis was uninfluenced on treatment with TiO_2 NPs, while growth (germination, root/ shoot length and biomass) was reduced at highest concentration. We report a decline in the net photosynthesis (A, E and gs), and enhanced oxidative and osmotic stress indicated by increased MDA and proline respectively due to the TiO_2 NP treatment. Results also suggest generation of ascorbate in order to metabolise ROS produced, probably to mitigate the oxidative damage.

MATERIALS AND METHODS

Plant materials and growth conditions

Oryza sativa (var. Jyoti), a high yielding, rice variety was obtained from Indian Council of Agricultural Research (ICAR), Goa and stored in dark at 4°C. TiO_2 NP (<25 nm size, Sigma-Aldrich) solution of 0, 2.5, 10, 50, 100 and 1000 ppm was prepared in Hoagland's solution (pH 6.5). Seeds were surface sterilized using 4% sodium hypochlorite (BDH) solution for 3 min, washed thoroughly with tap water and soaked for 3 days. 100 seeds were sown on germination paper in a Petri plate with respective concentration of TiO_2.

The seeds were germinated in a growth chamber equipped with lamps to provide an irradiance of 200 µmol $m^{-2}s^{-1}$ with 16 h photoperiod, temperature of 25 ± 2°C and relative humidity of 70- 75%. The 6 day old seedlings were transferred carefully in a hydroponic system containing respective concentration of TiO_2 NPs and were allowed to grow for a further 24 days. The hydroponic system ensured complete mixing of NPs by use of magnetic stirrers and proper aeration achieved by air pumps. The solutions were replenished every third day.

Nanoparticle Characterization Studies

TiO_2 NPs were obtained from Sigma-Aldrich Co. (USA). The physical characteristics as obtained from the supplier were: particle size (<25 nm), surface area (45-55 m^2g^{-1}), mp (1825 °C), and density (3.9 g mL^{-1} at 25 °C), containing 99.7% trace metals basis.

Size characterization using Scanning Electron Microscopy (SEM)

TiO_2 NP was characterized at 30000 X magnification using SEM (JSM 5800 LV, JEOL, Japan) at the National Institute of Oceanography, Goa.

Size characterization using X-ray Diffractometer (XRD)

X-ray Diffractometer (Rigaku) pattern of TiO_2 NPs was recorded at room temperature in the range of 2θ=20°-80° with a step of 0.02° and a speed= 2°/min using Cu kα radiation (λ=0.15406 nm).

Size characterization using dynamic light scattering (DLS) analyzer

DLS measurements in aqueous system were studied using dynamic light scattering ZetaPALS Analyzer (Brookhaven Instruments Corporation, New York, USA). Appropriate amount of TiO_2 NPs was suspended in distilled water as well as Hoagland's nutrient solution and was physically dispersed using an ultrasonicator for 2 min. Each measurement consisting of five replicates were measured with 10 s duration at 25°C and analyzed using BIC Particle Sizing Software Ver.5.23.

Germination, root and shoot length and biomass studies

Percent germination, root, shoot length was manually taken at the early stage (6^{th} day) of growth. The biomass was measured taking 10 plants from each TiO_2 NP treatment group after 30 days of growth. Fresh leaf tissue was dried at 75°C for 72 h noted as dry weight (d.w.).

Nanoparticle uptake

Uptake of TiO_2 NPs was determined using an inductively coupled plasma atomic emission spectrophotometer (Perkin-Elmer Optima 2000 DV ICP-OES) calibrated with single-element calibration standards. The ash was dissolved in 1% HNO_3 (Merck) solution and filtered through 0.45 µm Whatman filter paper. The filtered sample was used for ICP-OES analysis.

Photosynthesis measurements

Photosynthesis measurements were carried out using a portable infra-red gas analyzer (IRGA, ADC Bioscientific, LCi-SD, Hansatech, UK) according to Sharma and Hall (1996). The photosynthesis rate (A), transpiration rate (E) and stomatal conductance (gs) were measured at ambient temperature and carbon dioxide concentration and light intensity of 1200 µmol m^{-2} s^{-1} using a detachable light source provided by a dichroic lamp (Hansatech, UK).

Fluorescence measurements

Measurements were carried out using a fluorescence monitoring system (FMS, Hansatech instruments) according to Sharma *et al*. (1997) using Modfluor32 software. The maximum fluorescence (Fm), variable fluorescence (Fv) and the maximum quantum yield (Fv/Fm) ratio was calculated. The relative contributions for photochemical and non-photochemical energy dissipation measured as the photochemical quenching (qP) and non-photochemical quenching (qNP) were also calculated according to Schreiber et al (1986).

Extraction and analysis of photosynthetic Pigments

Plant tissue (0.1 g) was ground in 100 % Acetone (Merck HPLC grade) making a final volume of 1.5 ml and incubated overnight at 4°C. The homogenate was centrifuged at 4°C for 10 mins at 4000 x g. 1 ml of supernatant was collected and filtered through 0.2 µm Ultipor®N66®Nylon 6, 6 membrane

filter and 10 µl of sample was analyzed in a Waters HPLC system, according to Sharma and Hall (1996). The HPLC profile was extracted at 445 nm with a Waters 2996 photodiode array detector and peaks were identified based on RT and their spectral characteristics. *β*-carotene was used as an external standard for peak quantization.

Lipid per oxidation

Lipid peroxidation of the cell membrane was determined according to Sankhalkar and Sharma (2002). Peroxidation of lipids was measured as malondialdehyde (MDA) content using an extinction coefficient of 155 $mM^{-1}cm^{-1}$ at 532 nm.

Proline determination

Proline accumulation in fresh leaves was determined according to the method of Bates *et al*. (1973). L-Proline (Sigma ultra ≥ 99 % pure) was used as a standard for quantification by plotting a standard graph against concentration at 520 nm.

Ascorbate assay

Ascorbate (ASA) and dehydroascorbate (DHA) was estimated according to Kampfenkel *et al*. (1995). Ascorbic acid (sigma) was used as standard.

Statistical analysis

Data obtained was statistically analyzed using the procedure of ANOVA: Two-Factor without Replication and unpaired two-tailed student's t-test. Statistically significant difference '*a*' is reported when the $p < 0.05$.

RESULTS

Nanoparticle characterization

The TiO_2 NPs were characterized under scanning electron microscope (SEM) for their shape and was observed to be spherical to round (Fig 1a). X-ray diffraction (XRD) patterns were analyzed using Scherrer's equation and the average crystalline size was measured to be 15 ± 2 nm (Fig 1b). Using dynamic light scattering (DLS) analyzer, a narrow distribution of the particle size of nano-$TiO_{2\ powder}$ in Hoagland's solution was measured to be 23 ± 2 nm (Polydispersity of 0.074) and in distilled water was in the range of 12 ± 2 nm (Polydispersity of 0.191) which were in agreement with the XRD measurements. The sizes obtained were as specified by the supplier (TiO_2 NPs, size <25 nm; Sigma-Aldrich, USA). The TiO_2 powder resulted in a suspension that appeared cloudy and did not seem to settle over the sampling time.

Table 1 Percent germination (%G), Root and shoot length of rice plantlets (6 day old) treated with TiO_2 NP at different concentration.

TiO_2 NPs (in ppm)	% G	Root length (mm)	Shoot length (mm)
0	94.3 ± 0.9	51.31 ± 4.0	37.9 ± 1.7
2.5	93.1 ± 0.2	51.82 ± 2.6	36.4 ± 2.9
10	92.1 ± 1.4	53.04 ± 4.2	36.8 ± 2.9
50	90.9 ± 2.5	52.67 ± 1.7	36.3 ± 0.1
100	92.9 ± 2.5	47.93 ± 5.5	37.0 ± 5.3
1000	91.8 ± 1.4	39.29 ± 7.4	33.7 ± 8.7

Values are the mean (± S.D) of 7 replicates.

Percent germination and root/ shoot length measurements

The percentage germination was unaffected while shoot and root length decreased by approximately 11% and 23% respectively as a result of 1000 ppm TiO_2 NP treatment (Table 1). The shoot biomass (f.w. & d.w.) showed no change up to 100 ppm but decreased by approximately 9% at 1000 ppm (Table 2). The root biomass (f.w.) decreased slightly at higher concentration of TiO_2 NP (Table 2). However, in contrast to these results, the root biomass on dry weight basis increased by 476% at the highest concentration of TiO_2 as compared to control (Table 2). Plants grown at 1000 ppm of TiO_2 also showed necrosis at the leaf tips (data not shown).

Table 2 Biomass of rice plantlets (30 day old) treated with TiO_2 NP at different concentration.

	Biomass (g)			
TiO_2 NPs (ppm)	Shoot (F.W)	Shoot (D.W)	Root (F.W)	Root (D.W)
0	0.74 ± 0.03	0.076 ± 0.02	0.33 ± 0.03	0.026 ± 0.003
2.5	0.72 ± 0.09	0.075 ± 0.02	0.32 ± 0.02	0.028 ± 0.003
10	0.76 ± 0.07	0.075 ± 0.01	0.29 ± 0.05	0.021± 0.003
50	0.76 ± 0.01	0.075 ± 0.01	0.30 ± 0.01	0.023 ± 0.001
100	0.77 ±0.05	0.076 ± 0.02	0.30 ± 0.03	0.15 ± 0.02
1000	0.67 ± 0.15	0.069 ± 0.02	0.32 ± 0.13	0.13 ± 0.02

Values are the mean (± S.D) of 7 replicates.

Accumulation of TiO_2 NPs using ICO-OES

Plants showed a 3-fold increase in the Ti content up to 10 ppm as a result of the treatment (Table 3). Further increase in the TiO_2 concentration during treatment showed little difference in Ti content as observed in control shoots. Ti content in roots, however, showed a linear increase. 50 ppm TiO_2 NP treatment resulted in 3-fold increase in Ti content in roots, which was increased to 23.5 fold at 1000 ppm concentration (Table 3).

Table 3 Shoot and root Ti content of 30 day old plantlets exposed to TiO_2 NP treatment.

TiO_2 NP (ppm)	Ti content (mg/L)	
	Shoot	Root
0	0.9 ± 0.0009	0.9 ± 0.0003
2.5	3.0 ± 0.0034	1.1 ± 0.0224
10	3.0 ± 0.0038	2.0 ± 0.0073
50	0.9 ± 0.0015	3.1 ± 0.0295
100	1.0 ± 0.0028	21.2 ± 0.914
1000	1.2 ± 0.0062	21.3 ± 0.034

Values are the mean (± S.D) of 3 replicates.

Table 4 Carotenoid and chlorophyll content of TiO_2 NP treated rice plants at 0-1000 ppm concentration after 30 days of growth.

Pigments (conc. in µg)	Treatment with TiO_2 NP (in ppm)				
	0	2.5	10	100	1000
Neoxanthin	0.82	0.87	1.63	1.34	1.10
Violaxanthin	1.67	1.82	3.39	2.97	2.41
Antheraxanthin	0.25	0.31	0.50	0.50	0.32
Lutein	3.44	3.07	7.25	6.09	4.57
Chlorophyll *b*	6.72	6.77	13.54	11.87	8.93
Chlorophyll *a*	5.62	5.67	11.88	10.36	7.76
β-Carotene	1.51	1.32	3.61	3.09	2.17

Photosynthetic studies

Chlorophyll fluorescence indicative of light reaction of photosynthesis was not significantly affected on treatment with high concentration of TiO_2 NP (Fig 2). The photosynthetic efficiency of PSII, measured as F_v/F_m ratio and photochemical quenching was similar to that of control, however, a 12% increase in non- photochemical quenching (qNP) indicative of dissipation of absorbed light energy in the form of heat was seen as a result of TiO_2 treatment (Fig 2).

The rate of CO_2 exchange measured as net photosynthesis (A), transpiration rate (E) and stomatal conductance (gs) decreased at higher concentrations of TiO_2 NP treatment (Fig 3). A slight increase in the rate of net photosynthesis (11%), transpiration rate (46%) and stomatal conductance (70%) was observed at low concentration (2.5 ppm) of TiO_2 NPs (Fig 3), however, an

increase in the concentration of TiO_2 resulted in decreased photosynthesis, transpiration and stomatal conductance. Net photosynthesis declined to 53% at 1000 ppm of TiO_2 concentration as compared to control. Likewise, transpiration rate and stomatal conductance also declined to 77% and 66% respectively at 1000 ppm TiO_2 compared to control (Fig 3).

The HPLC chromatogram obtained at 445 nm showed no qualitative changes (Fig 4). However, quantitative changes in the pigments were observed (Table 4). Chlorophyll *a* and *b* as well as carotenoid content (neoxanthin, violaxanthin, antheraxanthin, lutein, and β-carotene) increased on treatment with TiO_2 NP as compared to control (Table 4). A linear increase of up to 5% in Chl *a*/*b* ratio was observed as compared to control at high concentration. The Chl: Car ratio also increased at low concentration (2.5 ppm) but was not significantly different from control at high concentrations.

Lipid peroxidation and Osmotic stress

The malondialdehyde (MDA) content indicative of osmotic stress did not change significantly up to 50 ppm, however, an increase of 29% at 100 ppm and above concentration of TiO_2 NP was observed (Fig 5a).

The treatment also resulted in osmotic stress indicated by the increase in proline content (Fig 5b). Proline level increased more than 2-fold as compared to the control as a result of the TiO_2 treatment (Fig 6b).

Content of ascorbate, a non-enzymatic antioxidant, was also determined and was found to increase by 260% as a result of 1000 ppm TiO_2 treatment (Fig 5c).

Figure Legends

Figure 1 Scanning electron micrograph of TiO_2 NP at 30000X magnification **(a)**, X-Ray Diffraction pattern of TiO_2 NP **(b)** recorded at room temperature in the range 2θ=20°-80° with a step of 0.02°, speed= 2°/min using Cu kα radiation (ʎ=0.15406 nm).

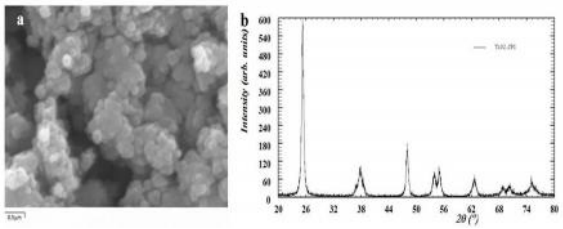

Figure 1 Scanning electron micrograph and XRD pattern of TiO_2 NP

Figure 2 Photosynthetic efficiency of photosystem II (Fv/Fm), photochemical quenching (qP) and non- photochemical quenching (qNP) of rice plants treated with TiO_2 NPs after 30 days of growth. The error bars represent the mean (± S.D) of 7 replicates. The statistical analysis was performed using the procedure of Students t-test, 'a' representing statistically significant values when $p < 0.05$.

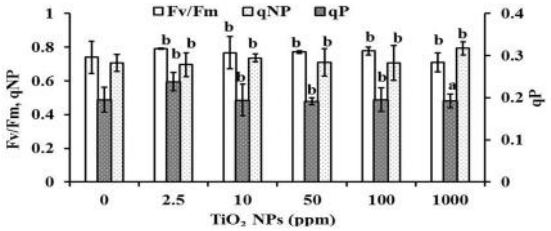

Figure 2 Light reactions of photosynthesis

Figure 3 Net photosynthesis rate (A), transpiration rate (E) and stomatal conductance (gs) of rice plants treated with TiO_2 NPs after 30 days of treatment. The error bars represent the mean (± S.D) of 7 replicates. The statistical analysis was performed using the procedure of Students t-test, 'a' representing statistically significant values when the $p < 0.05$.

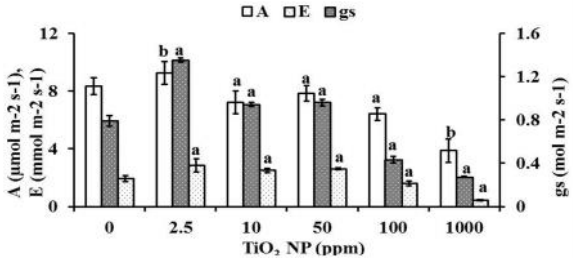

Figure 3 Dark reaction of photosynthesis

Figure 4 HPLC profile of carotenoid and chlorophyll content of rice plants at 445 nm treated with TiO_2 NP after 30 days of treatment [a= Control and b= 1000 ppm TiO_2 NPs].

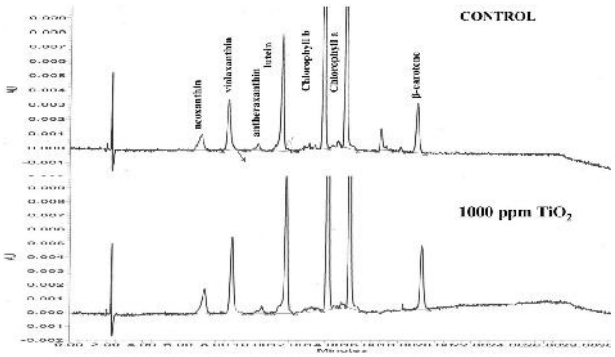

Figure 4HPLC chromatogram of photosynthetic pigments

Figure 5 The MDA content **(a)**, proline content **(b)** and ascorbate content **(c)** of rice plants treated with TiO_2 NP after 30 days of treatment. The error bars represent the mean (± S.D) of 7 replicates. The statistical analysis was performed using the procedure of Students t-test, 'a' representing statistically significant values when the $p < 0.05$.

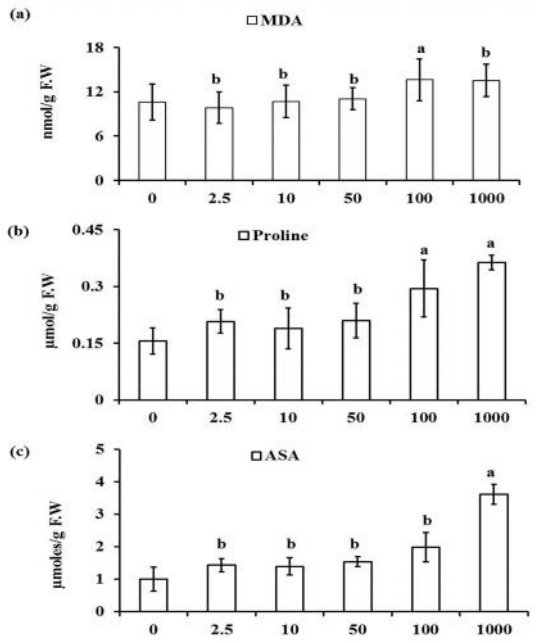

Figure 5 The MDA content **(a)**, proline content **(b)** and ascorbate content **(c)** of rice plants treated with TiO_2 NP

DISCUSSION

Our result showed no effect of TiO_2 NP treatment on seed germination of *Oryza sativa* var. Jyoti plants (Table 1). This may be due to the impermeability of rice seed coat to the nanoparticles till the radicle emerges. Wierzbicka and Obidzinska (1998) have also shown impermeability of seed coat to NPs. We also observed that the shoot and root length was uninfluenced up to 100 ppm, while toxicity was observed at highest concentration of TiO_2 NPs (Table 1). Shoot and root biomass showed no changes on fresh weight (f.w.) basis, however an increase in the root dry weight (d.w.) was observed at high concentration (Table 2). Increase in root d.w. probably may be as a result of significant accumulation of Ti in the plant roots treated with 100 and 1000 ppm TiO_2 NPs. Kurepa *et al*. (2010) reported that roots of *A. thaliana* released mucilage forming a hydrogel capsule either inhibiting or facilitating the entry of TiO_2 NPs. Watanabe (2008) reported that mucilage released may absorb, inactivate or accumulate toxic heavy metals in the rhizosphere depending on the plant species. Our data revealed massive accumulation of Ti particles in roots (Table 3), but little accumulation in shoots at high concentration. This accumulation indicates active exclusion of Ti by root cells in order to protect the shoots and more importantly the photosynthetic tissue. Ti accumulation has been reported in parenchyma of wheat root and vascular tissue (Laure *et al*., 2011) and roots and outer leaves of cabbage plants (Hara *et al*., 1976).

TiO_2 NPs did not show toxicity to light reaction of photosynthesis probably because it behaves more like metal oxides, rather than like free metal ions (Farrè *et al*., 2009) which are known to cause toxicity. In our study, no significant changes in the PSII quantum efficiency (Fv/Fm ratio) and photochemical quenching (qP) were observed (Fig 2), however, CO_2 fixation rate, transpiration rate and stomatal conductance declined significantly (Fig 3) at high concentration. Our results indicate that decrease in the net CO_2 fixation is probably a result of reduction in the stomatal conductance rather than metabolic limitation. TiO_2 NP treatment results in blocking pores in addition to decreasing root pores (Asli and Neumann, 2009). This may bring about lowering of water uptake causing osmotic stress, as observed in our study (Fig 5b). This osmotic stress could lead to stomatal closure which in turn would lower the CO_2 fixation. In contrast to our result, Yang and Watts (2005) have reported improved growth in spinach due to TiO_2 treatment, in addition to, better absorption of nitrate. This then accelerates the biosynthesis of proteins and chlorophyll and enhances photosynthesis and plant biomass. Lu *et al*. (2002) also observed that TiO_2 NPs increased nitrate reductase activity in soyabean. We observed an increase in photosynthetic pigments as a result of TiO_2 but negligible increase in shoot biomass or CO_2 fixation.

TiO_2 NPs are known to produce reactive oxygen species (ROS) due to its photocatalytic property (Hund-Rinke and Simon, 2006) resulting in lipid peroxidation, damaging DNA, proteins and lipids (Kelly *et al*., 1998). Increased levels of MDA observed in our study indicate oxidative stress and peroxidation of biological membrane (Fig 5a). Report by Ghosh *et al*. (2010) showed an increase in MDA content in *Allium cepa* as a result of TiO_2 NP treatment. They also reported geno-toxicity potential of TiO_2 NPs in both plant and human lymphocytes.

Exposure to heavy metals is known to cause water imbalance in the plants, suggesting that proline accumulation induced by metals depends on the development of metal-induced water stress in plant leaves (Chen *et al*., 2001). In our study, we observed that proline, an osmo-protectant increased with increase in TiO_2 NP concentration, indicating osmotic stress (Fig 5b). As a consequence of the osmotic stress, stomatal limitation was observed (Fig 3) which probably limited the CO_2 fixation and transpiration rate (Fig 3) as reported in this study.

ASA is an important component of the antioxidant defense system in plants serving as a reductant for the peroxidative removal of H_2O_2 (Noctor and Foyer, 1998). Increase in the non-enzymatic antioxidant, ascorbic acid (ASA) (Fig 6c), observed in this study was probably in order to mitigate, oxidative as well as osmotic stress induced by TiO_2 NPs. Guo *et al*. (2005) reported that ASA decreased the electrolyte leakage in rice roots subjected to Al toxicity and alleviated the inhibition of Al on root growth, indicating that ASA and its precursor increased the resistance of rice seedlings to Al toxicity. They also suggested that ASA decreased the H_2O_2 and MDA level in rice seedlings exposed to chilling, drought and Al stress. Our results infer that a significant increase in ascorbate content at high TiO_2 NP treatment (Fig 5c) caused a lesser amount of lipid peroxidation (Fig 5a) in *Oryza sativa* var. Jyoti.

CONCLUSION

Titanium dioxide nanoparticle effect (TiO_2 NPs) on plant growth (root, shoot length and biomass) and photosynthesis was investigated. TiO_2 NPs used in this study showed no effect on seed germination, root and shoot length and light reaction of photosynthesis (Fv/Fm ratio and qP), however, it decreased carbon dioxide assimilation (A), transpiration rate (E) and stomatal conductance (gs) in *Oryza sativa* var. Jyoti at high concentration. TiO_2 NP treatment resulted in an increased MDA, proline and ascorbate content at high concentration indicating oxidative as well as osmotic stress to plants. Overall, TiO_2 NPs caused little or no effect on light reaction of photosynthesis and pigment biosynthesis but led to a phytotoxic effect on the dark reaction of photosynthesis (carbon dioxide fixation).

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