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Enhancement of rice (*Oryza sativa* L.) physiological and yield by application of nano-titanium dioxide

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Abstract

Engineered nanoparticles (ENPs) are reported as potentially response to rice physiological and production. The research aimed to investigate the effects of suspended nano-titanium dioxide (sn-TiO_2), which is non-toxic to ecology and on the physiology and yield of Thai rice. Selected rice cultivars of RD41 were soaked and sprayed with three difference concentrations of sn-TiO_2 (T0.01, T0.02, T0.03 and C) through growing period. Tiller number per plants, stem height and leaf chlorophyll of rice RD41 cultivars were analyzed at tillering (40 days), flowering (70 days), and final harvesting (100 days), whereas the biomass and yield were evaluated at final harvesting (100 days). The results showed the concentration of T0.03 had highest effects on rice RD41 for all studies, which showed non-significance of differences at $p \leq 0.05$ compared to T0.02. Application of T0.01, T0.02 and T0.03 treatments increased total biomass 33.69, 42.66 and 47.91 g plant^{-1}, respectively, compared to control (30.49). Application of T0.02 enhanced the plant growth and caused increases in the yield of rice, which impacted food availability. According to the results obtained, the function of sn-TiO_2 played a positive role in many aspects. For instance, sn-TiO_2 could increase light harvesting to activate the photosynthesis rate of rice RD41. Besides, nitrogen metabolism was improved by sn-TiO_2 and stimulated protein and pigments content. Moreover, our observed decreasing in injury indices compared to the control group, which caused improvement in cell enlargement, cell elongation and plant growth. Atomic absorption spectrometric result ensured that there is no unforeseen Ti contamination in all part of rice. These findings are important supplementary factors to the application of sn-TiO_2 for the crop yield and quality with a proper concentration for their benefits potential.

Keywords: sn-TiO_2, Rice, Physiology, Yield, AAS.
Abbreviations: T0.01_titanium dioxide 0.01% w/v; T0.02_titanium dioxide 0.02% w/v; T0.03_titanium dioxide 0.03% w/v; C_control.

Introduction

The sn-TiO_2 is ultrafine material with the particle size in the range of 1-100 nm. Compare to the non-nanoscale sn-TiO_2 element, the nanomaterials exhibit a promising unique physical and chemical properties due to their large specific surface area. Increasing evidences in the literature, suggest that the sn-TiO_2 application are useful for plant protection. They also boost plants ability to take up other nutrients by enhanced total chlorophyll biosynthesis and enzymatic activities, resulting in high yield (Cigler et al., 2010; Kovacik et al., 2014; Lyu et al., 2017). Several studies have explained and proposed the mechanism of actions of Ti as a growth promoter for plants, including nitrogen fixation (Konishi and Tsuge, 1936) and their influence on plant metabolism by increasing absorption of nutrient elements (Simon et al., 1988), redox reactions (Carvajal et al., 1995) and stimulation of enzymatic activities and photosynthesis (Carvajal and Alcaraz, 1998). However, the plant response to sn-TiO_2 is still unclear and depends on the variation of different factors in environmental condition. Studies on biomass of crops have reported that plants uptake Ti ion by root uptake, leaf absorption and seed absorption (Lyu et al., 2017). Ti and Fe interact with one another, in which Ti can induce the expression of gene and enhance Fe uptake, when plant encounter Fe deficiency (Lyu et al., 2017). Moreover, the efficiency of root uptake of sn-TiO_2 depends on the particle size, which means root appears to be size selective (Tripathi et al., 2017). Wang et al. (2013) studied the effect of nanosized sn-TiO_2 on watermelon and reported that sn-TiO_2 entered leaf symplast through direct penetration. Hatami and Ghorbanpour, (2014) and Cox et al., (2016) soaked plant seeds in sn-TiO_2 and reported increase in nutrient absorption due to NPs penetration through seed coat, resulting in the enhancement of seed germination, root elongation and improved seedling growth. This research focus on replacing the conventional pesticide through the use of non-toxic sn-TiO_2 as a disinfection of seedling surface and foliar spray to improve the seedling growth, physiological and photosynthesis attributes of rice RD41. Recently, it has been indicated that sn-TiO_2 can influence plant growth and photosynthetic activity. The results revealed that sn-TiO_2 can significantly affect plant's biomass. Notably, low concentration of sn-TiO_2 caused significant improvement of plant nutrients, photosynthetic, anti-microbial and environmental-friendly over high concentration of sn-TiO_2 (Jaberzadeh et al., 2013). There are few scientific literatures addressing the issue of the application of sn-TiO_2 to directly promote the plant production and rate of photosynthesis. However, previous research has shown that sn-TiO_2 enhanced crop yield and suppress plant disease. Theerakarunwong and Chuaychai (2013) reported the efficacy of sn-TiO_2 nanoparticles on the activity of antioxidant in Mungbean. The conclusions obtained from these studies found that surface sterilization of explants with sn-TiO_2 instead of high toxicity sodium hypochlorite (NaOCl) and mercury chloride ($HgCl_2$) was successfully achieved, while sn-TiO_2 was not toxic to plant tissue. Moreover, nano sn-TiO_2 increases photosynthesis and plant growth. The other research has reported that sn-TiO_2 could greatly decreased infection of leaf and pea pod of *Vigna unguiculata*. Under UV irradiation, sn-TiO_2 induced the production of reactive oxygen species (ROS), which correlated to cell damage, by destruction of cell membrane and DNA damage (Owlade and Ogunleti, 2008). The report presented the potential influence of sn-TiO_2 on the yield of chickpea under cold weather since sn-TiO_2 inhibited cell metabolisms in plants, leading to reduce cell damage and increased the chlorophyll A, chlorophyll B and carotenoid contents (Mohammadi et al., 2014). Considering the above mentioned impact of sn-TiO_2 nanoparticles on physiological properties, the aims of this study was focused on the potential impacts of sn-TiO_2 on rice growth and performance. The results of this study can contribute to increase crop yield against different stresses.

Results and Discussion

Effect of sn-TiO_2 on the tiller number and stem height of rice RD41

Tiller number of rice RD41 was much higher than control group, which was treated with T0.03 by 8.17, 8.33 and 9.00 tiller per plant. Unlike control, plants showed lower tillers per plant were slightly increased by 7.00, 7.33 and 7.50 tiller per plant at T0.01, T0.02, T0.03, respectively (Table 1). The tiller per plant of both treated T0.01 and T0.02 of rice RD41 showed no significant difference, when compared with control group. The observed increasing tiller number was due to photosynthetic rate and yield as shown in Table 1. The T0.03 treatment showed significant effect in plant growth. The stem height of rice RD41 at tillering stage was 89.42 cm, while the stem height of the control, T0.01 and T0.02 were 85.92, 85.33 and 84.50 cm, respectively. At final harvesting stage, treatments showed no significant difference by 112.83, 113.50, 115.83 and 118.50 cm (Table 1). Plant growth and cell enlargement under proper concentration of sn-TiO_2 can be attributed to nitrogen metabolism by modulating inorganic-to-organic conversion rate (Karami and Sepehri, 2018)

The effect of Sn-TiO_2 on leaf chlorophyll of rice RD41

The total chlorophyll content of plants was increased under tillering and flowering stage, whereas under final harvesting stage, total chlorophyll was decreased. The net chlorophyll content at all stages was as following: C<T0.01<T0.02<T0.03. For example, total chlorophyll in leave under tillering stage was found to be 16.19, 23.03, 28.82 and 38.84 mg Chl/g fresh weight, respectively (Table 2). For spinach, bulk sn-TiO_2 boosted total chlorophyll and rubisco activity, leading to the increasing photosynthesis rate (Linglan et al., 2008). These results are in agreement with some other findings indicating that sn-TiO_2 is beneficial to cucumber photosynthesis (Cui et al., 2009). Moreover, TiO_2 nanoparticles induce chlorophyll and carotenoid contents by inhibiting those pigments from reactive oxygen species leading to capture more sunlight for photosynthesis (Mohannadi et al., 2014; Karami and Sepehri, 2018). Notably, this enhancement yield by sn-TiO_2 directly linked to suppress plant disease and also to the potential nutritional uptake (Servin et al., 2015; Mohammadi et al., 2014).

The effect of sn-TiO_2 on the panicle number of rice RD41

The results showed that panicle number of rice cultivars RD41 was increased after germinated with the suspension of sn-TiO_2 in plastic pots compare to the control group. The average of panicle number of rice cultivars RD41 at 100 days were 6.17, 6.33, 7.17 and 7.67 panicle per plant under C, T0.01, T0.02 and T0.03), respectively. For 3 treatments, panicle per plant was increased by 2.70, 16.22 and 24.32%, as compared with the control. The concentration of both T0.02 and T0.03 sn-TiO_2 caused more positive effect than T0.01 and control group, with no significant difference. The sn-TiO_2 at high concentration trends to exhibit more positive effect on seed germination, tiller number, dry mass, etc. The toxicity induced by higher concentration (0.03 %w/v) may have been produce the more toxic compounds, which is very important factor to consider during the study of Ti on plants. These results are consistent with Zahra et al. (2017), who have shown that sn-TiO_2 had positive impact on rice growth performance and reduce the infection of rice. Moreover, Owolade et al. (2008) reported that sn-TiO_2 enhanced the yield of cowpea by spraying method, which is relevance to chlorophyll content, leading to increase in photosynthesis rate.

The effect of sn-TiO_2 on the biomass of rice RD41

Dry biomass of shoot, root and total biomass were increased by sn-TiO_2. Upon control, T0.01, T0.02 and T0.03, total biomass was increased 30.49, 33.69, 42.66 and 47.91 g per plant, respectively (Fig 1). Total biomass of rice RD41 was obviously increased by T0.02 and T0.03. In contrast, no significant increases in total dry biomass were observed upon T0.01 and control group. In support of this finding, a recent study in wheat treated with bulk sn-TiO_2 at the concentration of T0.01-T0.03 sn-TiO_2 indicated that bulk sn-TiO_2 induced wheat biomass. TiO_2 affects plant biomass status by a combination of enhancing plant nutrition level and reducing disease presence, leading to an increased photosynthetic rate (Owlade and Ogunleti., 2008; Servin et al., 2015).

The effect of sn-TiO_2 on the panicle weight of rice RD41

The panicle weight of RD41 dry weights at age 100 days were 3.30, 3.74, 4.21 and 4.44 g (C, T0.01, T0.02 and T0.03) respectively (Table 3), indicating a percentage as follows; 13.32%, 27.57% and 34.47% (T0.01, T0.02 and T0.03), respectively, compared with the control group. Again, the

Table 1. Tiller number per plan and stem height of rice RD41. Rice samples soaked and sprayed with sn-TiO_2 for 100 days; T0.01, T0.02, T0.03 and C. The data represent the mean±SE. Different letters indicate significant differences among treatments at $p \leq 0.05$.

	Days of exposure sn-TiO_2	C	T0.01	T0.02	T0.03
Tiller number per plan	40 days	7.00±0.37b	7.33±0.33ab	7.17±0.31ab	8.17±0.40a
	70 days	7.33±0.42a	7.50±0.43a	7.33±0.49a	8.33±0.33a
	100 days	7.5±0.34b	7.67±0.33ab	7.67±0.49ab	9.00±0.63a
Stem height (cm)	40 days	85.92±0.99b	85.33±1.20b	84.50±1.02b	89.42±0.84a
	70 days	107.83±1.53ab	107.05±1.73b	111.50±2.70ab	114.27±2.20a
	100 days	112.83±1.78a	113.50±0.92a	115.83±2.85a	118.50±1.43a

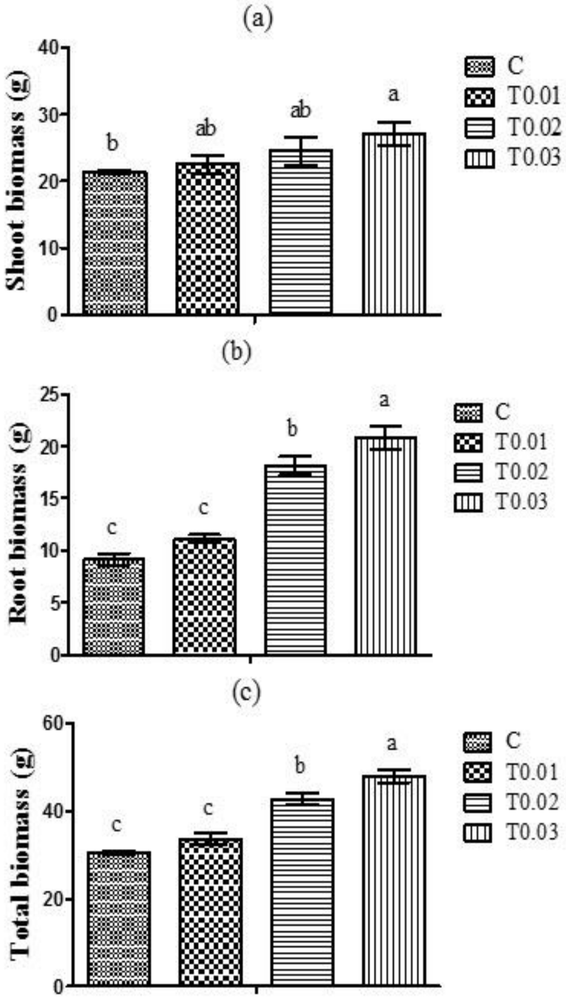

Fig 1. Shoot biomass (a), root biomass (b), and total biomass (c) of rice RD41. Rice samples by soaked and sprayed with sn-TiO_2 for 100 days; T0.01, T0.02, T0.03 and C. The data represent the mean±SE. Different letters indicate significant differences among treatments at $p \leq 0.05$.

Table 2. Leaf chlorophyll of rice RD41. Rice samples by soaked and sprayed with sn-TiO_2 for 100 days; T0.01, T0.02, T0.03 and C. The data represent the mean±SE. Different letters indicate significant differences among treatments at $p \leq 0.05$.

	Days of exposure sn-TiO_2	C	T0.01	T0.02	T0.03
Chl a	40 days	9.73±1.67b	12.83±1.35ab	14.71±0.61a	16.52±0.37a
	70 days	11.07±2.23b	14.50±0.13ab	15.66±0.06a	17.50±0.55a
	100 days	11.61±0.18b	12.22±0.90b	12.93±0.35b	14.91±0.42a
Chl b	40 days	6.46±0.99b	10.20±3.05b	14.11±2.44b	22.33±2.08a
	70 days	4.40±2.54b	2.34±1.35a	1.75±1.01a	4.28±2.47a
	100 days	7.54±0.31b	8.82±1.34b	10.02±0.74b	15.47±1.52a
Total Chl	40 days	16.19±2.65c	23.03±4.35bc	28.82±3.04ab	38.84±1.91a
	70 days	20.84±4.73b	32.86±1.47a	35.31±1.07a	39.74±2.56a
	100 days	19.15±0.49b	21.04±2.24b	22.95±1.09b	30.38±1.95a

Table 3. Panicle number per plant, panicle weight, fill grain, unfilled grain and percent fill grain per total grain of rice RD41. Rice samples soaked and sprayed with sn-TiO_2 for 100 days; T0.01, T0.02, T0.03 and C. The data represent the mean±SE. Different letters indicate significant differences among treatments at $p \leq 0.05$.

yield	C	T0.01	T0.02	T0.03
Panicle number	6.17±0.40b	6.33±0.21b	7.17±0.48ab	7.67±0.33a
Panicle weight	3.30±0.21b	3.74±0.37ab	4.21±0.14a	4.44±0.30a
Number of filled grain per panicle	105.32±7.78c	114.73±8.43bc	133.57±6.09ab	147.89±11.69a
Number of unfilled grain per panicle	22.02±3.91a	20.50±3.43a	19.70±2.74a	17.77±3.18a
Percent of filled grain per panicle	81.48±2.28a	84.05±1.85a	85.93±2.04a	87.76±1.88a

concentration of both T0.02 and T0.03 cause more positive effect than T0.01 and control group, showing no significant difference. The positive effects might be only based on the chelated Ti compounds which enhanced chlorophyll biosynthesis and enzymatic activities and increased photosynthesis and nutrient uptake, when applied to soils or onto leaves resulting in increased plant biomass or crop yield (Lyu et al., 2017).

Number of filled grain, unfilled grain and percent of filled grain per panicle

The number of filled grain was 105.32, 114.73, 133.57 and 147.89 grain per panicle (C, T0.01, T0.02 and T0.03), respectively, (Table 3), showing 8.93%, 26.82% and 40.42% (T0.01, T0.02 and T0.03), respectively, compared with the control group. Additionally, sn-TiO_2 could offer more filling gain. This approach would decrease the number of unfilled gains, leading to increase in percentage of filled gain over the control group by 81.48%, 84.05%, 85.93% and 87.76% (T0.01, T0.02 and T0.03, respectively). Some studies revealed that how nanoparticle coating may cause positive results on physiology and biomass of plant. For example, application of 0.04% Ti increased total yield of wandflower corms by 20% (Marcinek and Hetman, 2008). Many studies have observed and explained that Ti enhanced the growth of plant by participation in plant metabolism as a redox catalyst (Traetta-Mosca, 1913) and accumulated in as simulation organs (Geilmann, 1920). Moreover the optimal levels of Ti caused increased plant growth rate and reflected the intensity of chlorophyll content (Nˇemec and Káš, 1923).

Ti content of digested plants

The digested samples were analyzed using flame atomic absorption spectrometer for the determination of Ti at the wavelength of 298 nm. The concentrations of Ti in the digested supernatant were recorded and calculated with the external calibration. From the result, the level of Ti was non-detectable indicating that the RD41 cultivar was not contaminated after treatments. It is nothworthy to know that the germination, soaking and spraying with a suspension of sn-TiO_2 were free from Ti contamination and, hence, safe for human and environment.

Materials and Methods

sn-TiO_2 material

Anatase sn-TiO_2 nanoparticles with about 20 nm particle size were synthesized and characterized following our previous work (Theerakarunwong and Phanichphant, 2015). There concentrations of T0.01 (0.01 %w/v), T0.02 (0.02 %w/v) and T0.03 (0.03 %w/v) were suspended in water for 15 min in ultrasonic bath before germination.

Rice cultivar, germination and growth

Seeds of rice (*Oryza sativa* L.) cultivars RD41 immersed in 3 ranges of TiO_2 concentration by 0.01 %w/v, 0.02 %w/v and 0.03 %w/v, compared with a control group. Then, the germinated seeds were placed in plastic trays overnight to enhance seed germination and further growth in 12 inch plastic pots with 10 mL suspension sn-TiO_2 for 100 days. The control group was grown in the pots with no sn-TiO_2. Rice cultivar RD41 was soaked and sprayed with 3 different concentrations of TiO_2 every 10 days for 3 replicates. Tiller number per plants, stem height and leaf chlorophyll were evaluated during the growing period of rice at tillering (40 days), flowering (70 days), and final harvesting (100 days), including the biomass, yield and final harvesting (100 days). Besides, final harvesting was subjected to AAS to confirm the Ti contaminated for all part of rice.

Chlorophyll

Approximately 0.1 g of tissue was ground with a pestle and mortar in 10 ml of 80% acetone. The homogenate was centrifuged then the absorbance was measured at 663 and 647 nm in a spectrophotometer. Chlorophyll a and b were calculated using the equations provided by Lichtentalter (1987).

Acid digestion

1.0 g of dried root, leaf and stem were acid digested with 5 mL concentrated HNO_3 and 0.5 mL $HClO_4$ overnight. The extracts were then further digested for 20 min at $150^{o}C$ until a transparent supernatant was formed, followed by heated at the temperature to $225^{o}C$ for another 10 min. After cooling, the supernatant was filtered through Whatman filter paper no. 42 and the specified distilled water was added to obtain 5 mL transparent supernatant until further analysis by AAS. Total Ti content was determined using AAS as described below.

AAS analysis

Dried rice tissues were analyzed for Ti content by AAS (Perkin-Elmer) equipped with nebulizer with air/acetylene flame. The concentration of Ti metal ions in the sample was investigated by its absorbance using AAS software with the external standard calibration curve. Ti element was determined by absorption/concentration mode compare to the digested blank solutions. Three replicate determinations of Ti were conducted on each sample.

Statistical analysis

The ANOVA analysis was carried out and significances of differences were tested at $p \leq 0.05$ between control and treatment. The group differences were analyzed by Duncan's New Multiple Range Test (DMRT).

Conclusion

The suspension of sn-TiO_2 increased biomass, chlorophyll content, growth, and yield of the rice cultivars RD41. The results of analysis showed that sn-TiO_2 can enhance plant metabolism and cell division. Chlorophyll content of sn-TiO_2 treated plants showed significant increase by the activation of photochemical reaction of rice. The concentration of 0.02 and 0.03 %w/v was considered the highest affecting the plant growth and caused increases in the yield of rice. However, 0.02 %w/v sn-TiO_2 should be selected as the optimal concentration to enhance rice production, which is much safer than higher concentrations of Ti. Hence, sn-TiO_2 treatments by soaking and spraying can increase biomass and quality of rice cultivars RD41. Moreover, Ti element was not detected for all concentration treated in dried sample examined by AAS. This finding can be used as information on the potential influence of TiO_2 material on yield and antioxidant defense mechanism of crops.

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