Chapter 2

Application of Nanotechnology in Agriculture: Assessment of TiO_2 Nanoparticle Effects on Barley

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Additional information is available at the end of the chapter

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Abstract

Many aspects associated with the application of nanotechnology to agricultural activities are still unknown. In particular, there is not enough information on nanotoxicology in crops and we do not know the fate of nanoparticles in crops. Multiple experiments were carried out to study the effects of titanium oxide nanoparticles (nTiO$_2$) on barley (*Hordeum vulgare*). Germinating seeds were exposed to 0, 500, 1000, and 2000 mg l^{-1} nTiO$_2$. Seed germination percentage, mitotic index, root elongation, and Ti concentration in seedlings were observed. In a greenhouse experiment, plants of barley were grown to physiological maturity in control soil and soil enriched with 500 and 1000 mg nTiO$_2$ mg kg^{-1}, respectively. The duration of the growth cycle and the plant biomass was influenced by nTiO$_2$ compared to control plants. Concentrations of Ti were not very high with the exception of roots. However, the nTiO$_2$ soil amendment had an impact on composition and nutritional quality of barley grains. Concentrations of Ca, Mn, and Zn in kernels were increased by nTiO2 treatments. Concentration of amino acids was affected by the treatments as well. nTiO$_2$ treatments have the potential to influence the food chain and processing and economics of barley.

Keywords: nanotechnology, titanium dioxide nanoparticles, agriculture, crop growth

1. Introduction

Nanotechnology is a multidisciplinary field, which includes a wide range of processes, materials, and applications. The main aims of this new discipline are the characterization, fabrication, and manipulation of material at nanoscale level [1]. The reason why these new materials are so widely used is linked to their unique and novel properties principally related with the increase of the surface area to volume ratio. Nowadays, a lot of products are based on nanotechnology; at the beginning, these materials were applied in construction materials, new devices and techniques in

electronics, cosmetics, sporting equipment, wastewater treatment, medicine, and more recently in agriculture and the food industry [2]. The agri-food was the last sector in terms of succession to be interested by this technological revolution but at the same time it would be far reaching in the next years [1]. In fact, the nanotechnology has recently emerged as the technological advancement to develop and transform the entire agri-food sector, in terms of increasing global food production and nutritional value, quality and safety of food [3]. The type of nanoparticles (NPs) or nanomaterials (NMs) used in plant science are quite wide, but they could be clustered in two principal groups: the carbon nanomaterial (CBNMs) and the metal-based nanomaterials (MBNMs) [4]. In the group of MBNMs, the most common NPs are TiO_2, CeO_2, Fe_3O_4, ZnO, and $AgNO_3$ [5]. Our experiments were focused on TiO_2 nanoparticles ($n$$TiO_2$) that represent the most used nanomaterial between the MBNMs. Several papers demonstrate the positive effects of $n$$TiO_2$ on plants [6–9]. More recently, Dehkourdi and Mosavi [10] used nano-anatase to treat parsley seeds, which resulted in an increase in the percentage of germination, the germination rate index, the root and shoot length, the fresh weight, the vigor index, and the chlorophyll content of the seedlings. Also, Feizi et al. [11] observed that the germination rate of *Salvia officinalis* improved when the seeds were exposed to $n$$TiO_2$. Previous studies demonstrate a positive effect also during the plant vegetative growth, for example, Hong et al. [7] demonstrate an acceleration in the rate of evolution of oxygen by chloroplasts in spinach plants. Another experiment on spinach demonstrated a gain in the photosynthetic carbon reaction in treated plants [9]. More recently, Qi et al. [12] treated tomato plants with $n$$TiO_2$ and put them in a mild heat stress, the plants resulted to have an improvement in the photosynthetic rate with respect to the control ones. Currently, the application of nanomaterials in the field of primary production is still under investigation, and therefore, it may take many years before specific nanoproducts for agriculture are commercialized worldwide [1]. Since the studies performed up to now have been conducted in a very simplified experimental condition, we still lack accurate information on what is happening in the soil. Further research is required to ensure complete success for these applications of nanotechnologies [13].

2. Materials and methods

2.1. Nanoparticle characterization

Titanium (IV) oxide anatase nanopowder having a minimal average particle size of 25 nm was purchased from Sigma-Aldrich (product ID 637254). Titanium nanoparticle ($n$$TiO_2$) characterization was carried out at the Facility for Environmental Nanoscience Analysis and Characterization (FENAC), University of Birmingham (UK). Further details on analytical methods are provided by Marchiol et al. [14].

2.2. Laboratory experiment

2.2.1. Seed germination and root elongation

Ten seeds of spring barley (*Hordeum vulgare* L. var. Tunika) were transferred in sterile conditions into each Petri dishes soaked with distilled water (Ctrl), 500, 1000, and 2000 mg l^{-1} $n$$TiO_2$

suspensions; each treatment was replicated three times. The Petri dishes were taped and placed in the dark at 21°C for 3 days. The germination percentage was calculated as the ratio of germinated seeds out of the total seeds. The seedlings obtained were used for the measurements of their total root length with ImageJ [15]. Root elongation was calculated as the average length and the sum of all roots emerged from each seed.

2.2.2. Mitotic index

Seeds of barley were sterilized and transferred in sterile conditions into Petri dishes soaked with distilled water. After 3 days, the germinated seedlings with actively growing roots (at least 2.5 cm in length) were placed in the $n\text{TiO}_2$ suspensions (0, 500, 1000, 2000 mgl^{-1}) for 24 h. Ten root tips per each treatment were studied to evaluate possible genotoxic effects of $n\text{TiO}_2$. The samples prepared were evaluated for a total of about 10,000 cell observations per treatment. The mitotic index was recorded in Feulgen-stained preparations as the percentage of dividing cells out of the total number of cells scored.

2.2.3. Titanium uptake

The treated barley seedlings were rinsed three times with MilliQ water. Subsequently, they were divided into three portions: roots, seeds, and coleoptiles. The seedlings portions were dried at 70°C for 24 h, and they were acid-digested (10 ml HNO_3 65%) in a microwave oven according to USEPA method 3052. Plant extracts were filtered (0.45 mm PTFE), diluted with MilliQ water, and analyzed by ICP-OES.

2.3. Greenhouse experiment

2.3.1. Soil characterization and $nTiO_2$ amendment

The possible effects of $n\text{TiO}_2$ to barley were also evaluated along their entire life cycle; for this purpose, seeds of barley were grown in soil contaminated with $n\text{TiO}_2$ at 500 and 1000 mg kg^{-1}. The soil characterization data were reported by Marchiol et al. [14]. According to Priester et al. [16], the soil was amended with $n\text{TiO}_2$ powder before sowing plants reaching the final concentration of 500 and 1000 mg kg^{-1} of $n\text{TiO}_2$.

2.3.2. Plant growth

In a semi-sealed greenhouse, seeds of spring barley were sown in pots containing the $n\text{TiO}_2$-enriched soils. After 2 weeks, seedlings were thinned to two seedlings per pot. During the plant growth, the pots were watered twice a week to maintain soil at 60% WHC. Phenological stages were monitored by adapting the Decimal Growth Scale [17] throughout the growth cycle and were based on 50% of plants within the treatments at each stage. Plants were harvested at physiological maturity. Plant shoots were severed at the collar and separated into stems, leaves, spikes, and grains. Leaf area was measured using a LI-3100C Area Meter. The plant fractions were oven dried at 105°C for 24 h and weighed.

2.3.3. Spectroscopy analysis

Plant fractions were acid-digested in a microwave oven according to USEPA method 3052. Titanium concentration in plant fractions, such as roots, stems, and leaves, was determined by an ICP-OES, whereas Ti concentration in kernels was determined by an ICP-MS.

2.3.4. TEM observations

Serial ultrathin sections from each species were cut with a diamond knife, mounted on copper grids, stained in uranyl acetate and lead citrate, and then observed under a Philips CM 10 transmission electron microscope (TEM) operating at 80 kV.

2.3.5. Macronutrient and micronutrient concentrations in kernels

Total B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, and Zn contents were determined by an ICP-OES with an internal standard solution of Y. Total Ce and Ti contents were determined by an ICP-MS with an internal standard solution of ^{72}Ge and ^{89}Y. Total N and S content were determined through an Elemental CHNS Analyzer using up to 2.5 mg of finely ground samples.

2.3.6. Amino acids in kernels

Amino acids analysis was performed using a LC 200 Perkin Elmer. More technical details about amino acids analysis were provided by Pošćić et al. [18].

2.3.7. Data analysis

The experiments were carried out in a completely randomized factorial design. Analysis of variance was conducted with a one-way ANOVA. Tukey's Multiple Comparison test at 0.05 p level was used to compare means. Statistical analysis was performed using the SPSS program (SPSS Inc. Chicago, IL, USA, ver. 17).

3. Results

3.1. Characterization of *n*TiO_2

The *n*TiO_2 powder measured with BET has a specific surface area equal to 61.6 $m^2 g^{-1}$ that average value is slightly higher than the product specifications which declares a specific surface area comprehended between 45 and 55 $m^2 g^{-1}$. The height distribution measured with AFM of the *n*TiO_2 powder results 41.8 ± 24.3 nm on average (**Figure 1A**). The *n*TiO_2 suspension was characterized at dimensional level also by TEM (**Figure 1B**). In this case, the instrument allows to measure the diameters of nanoparticles and the average size result equal to 24.09 ± 7.22 nm. The DLS instrument gives information about nanoparticles at three different levels: (i) at size level, in fact the instrument displays the zeta average size of nanoparticles, which correspond to the average diameter; (ii) at stability level by measuring the zeta potential of nanoparticles,

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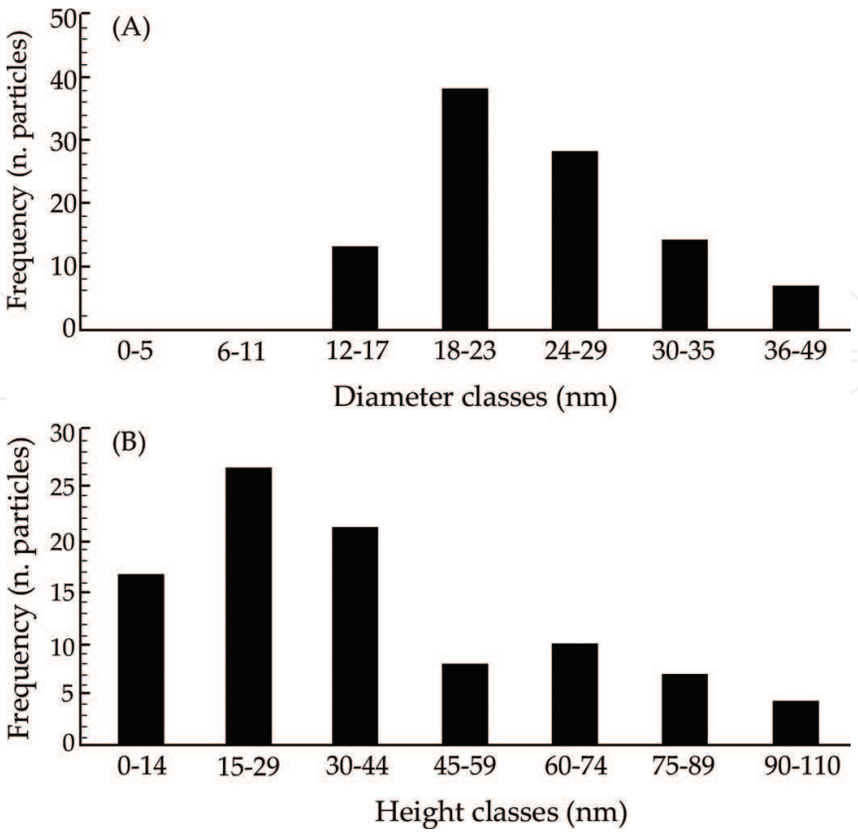

Figure 1. Characterization of: (A) height classes (nm) of nTiO$_2$ in powder form by AFM. (B) Diameter classes (nm) of nTiO$_2$ suspension obtained by TEM.

this index gives information about the electric potential in the interfacial double layer, if this value is comprised between −30 and +30 mV, the suspension tends to flocculate; and (iii) at heterogeneity level by the Polydispersity Index (PDI). The nTiO$_2$ suspensions result to have a zeta average size, zeta potential, and PDI equal to 925 ± 105 nm (**Figure 2A**), 19.9 ± 0.55 mV (**Figure 2B**), and 0.84 ± 0.17 nm, respectively. These values indicate a suspension made by big nanoparticles which tend to aggregate along time, and this brings to a wide-size distribution.

3.2. Seed germination experiments

3.2.1. Germination and root development

After 3 days, the treated seeds (**Figure 3A**) were used for the calculation of germination percentage (**Figure 3B**). The root elongation does not seem affected by nTiO$_2$ treatments at 1000 and 2000 mg l^{-1}, in fact, the average values are quite comparable with the germinated control seeds, whereas the average total roots value seems to slightly increase with increasing concentrations. The statistical analysis, however, shows there are not significant differences between treatments. The germinated seeds treated for 7 days were used for measuring their root elongation (**Figure 3C**). The root elongation does not seem affected by nTiO$_2$ treatments at 1000 and 2000 mg l^{-1}; in fact, the average values are quite comparable with the germinated control seeds,

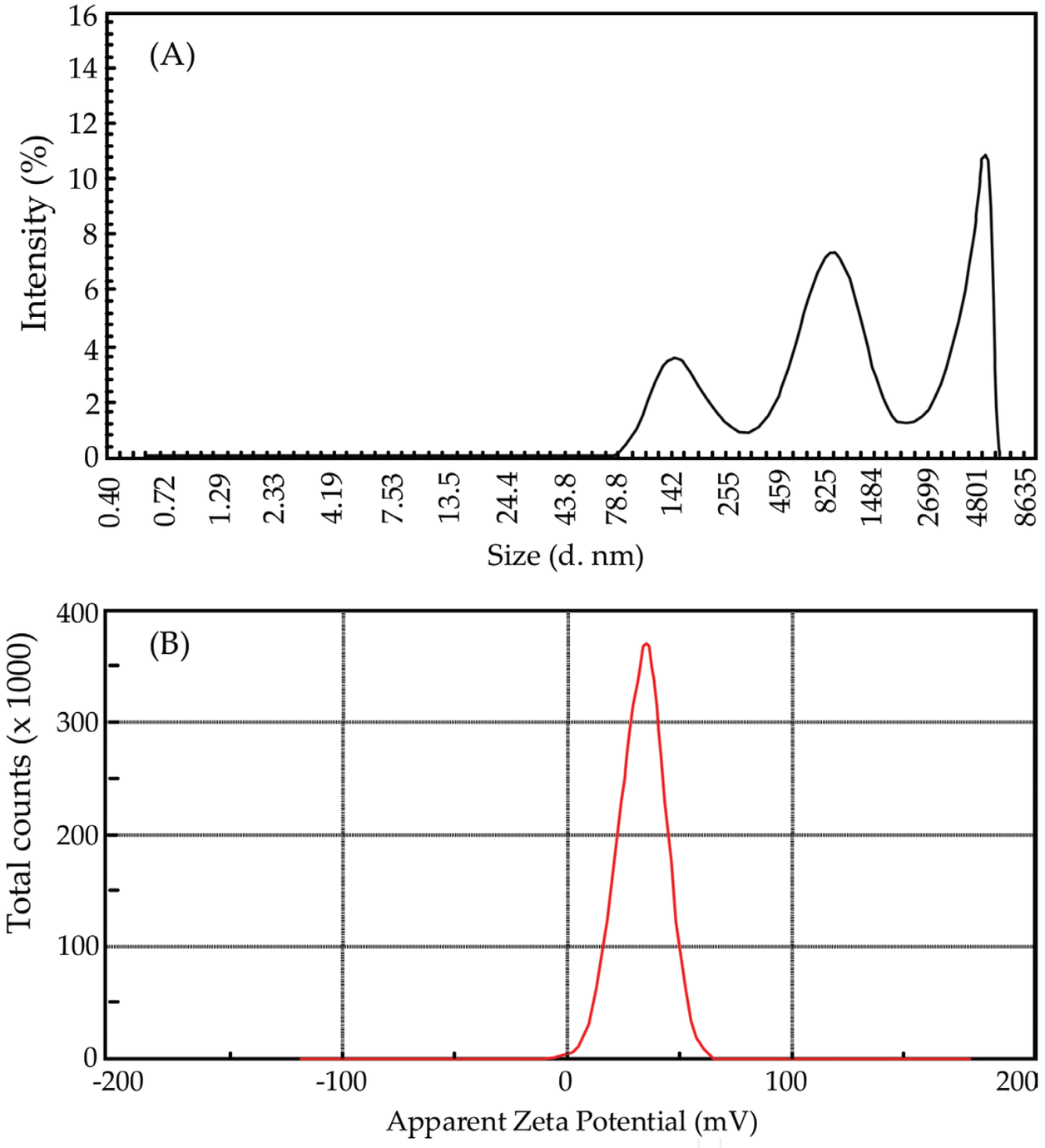

Figure 2. DLS data of $n\text{TiO}_2$ suspension: (A) $n\text{TiO}_2$ zeta average size distribution. (B) $n\text{TiO}_2$ zeta potential.

rather the average total roots value seems to slightly increase with increasing concentrations. Conversely, the seeds treated with $n\text{TiO}_2$ at 500 mg l^{-1} seemed to be affected in a negative way with respect to the other treatments. The statistical analysis has put on evidence a significant negative effect of $n\text{TiO}_2$ at 500 mg l^{-1} for the root elongation, instead there are no significant differences between the other treatments.

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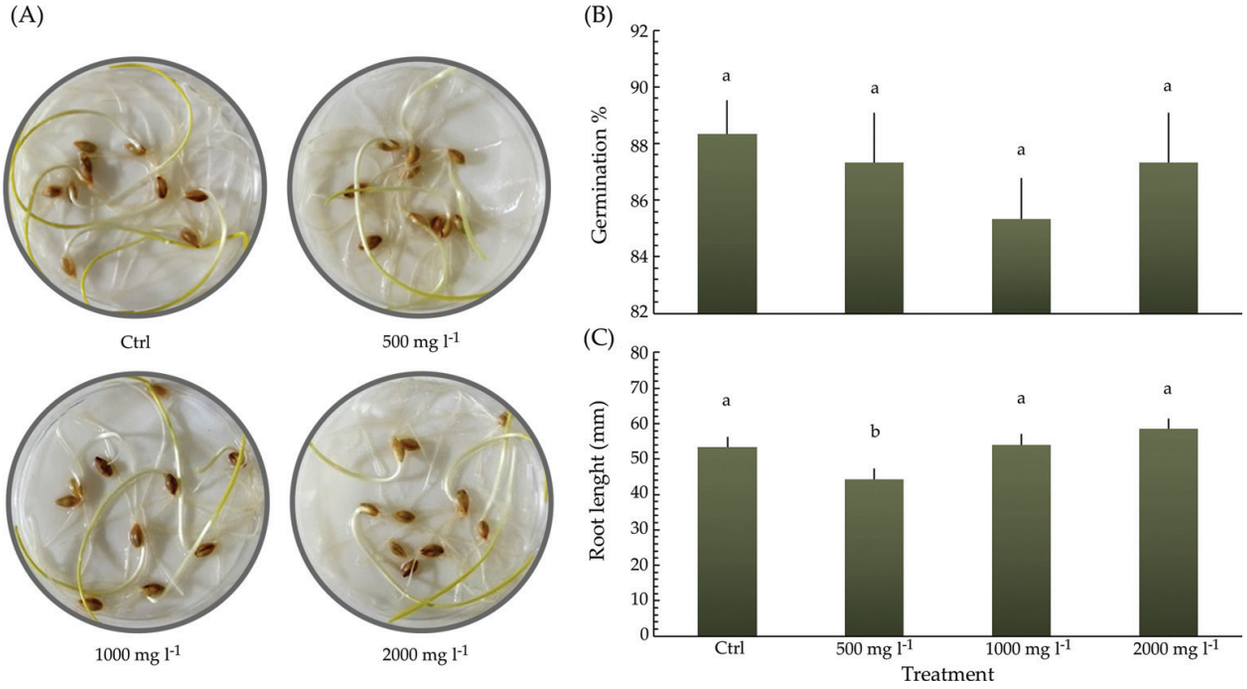

Figure 3. (A) Petri dishes with treated barley seedlings; (B) germination percentage of seeds (mean ± SE; $n = 3$); and (C) total root length in barley seedlings treated with $n\text{TiO}_2$ suspension at 0, 500, 1000, and 2000 mg l^{-1}.

3.2.2. Mitotic index

Figure 4A reports data of mitotic index (MI), which was used as a sensor of genotoxicity. As plant roots grow, the cell division is usually very fast in the apical meristem of root tips. In our case, the control seedlings have a MI lower than the seedlings treated with $n\text{TiO}_2$ suspension at 500 mg l^{-1} but comparable with the other treatments. Like the germination percentage also this parameter results not significantly affected by treatments.

3.2.3. Titanium seedlings uptake

The concentration of total titanium in different portions of barley seedlings is shown in **Figure 4B**. A dose-response was recorded in the accumulation of titanium since the titanium concentration in the seedling fractions increased with the increase of $n\text{TiO}_2$ exposure concentration. In particular, the seedlings grown in the presence of $n\text{TiO}_2$ at 500 mg l^{-1} did not uptake and translocate the titanium in other seedling portions. Instead, the seedlings grown in the presence of $n\text{TiO}_2$ at 1000 mg l^{-1} showed an uptake and a translocation of titanium in each portion. This trend is confirmed by the seedlings grown in the presence of $n\text{TiO}_2$ at 2000 mg l^{-1}; in fact, these seedling portions have the highest concentrations of titanium with respect to the seedling portions of the other treatments. The roots are the most interested area of accumulation; in fact, this portion recorded the highest concentrations of total titanium than the other portions for each treatment. This is particularly evident in the seedlings grown with $n\text{TiO}_2$ at 1000 mg l^{-1} where the concentration results significantly different from the other seedling portions, whereas it is not like that in seedlings grown in the solution with 2000 mg l^{-1}.

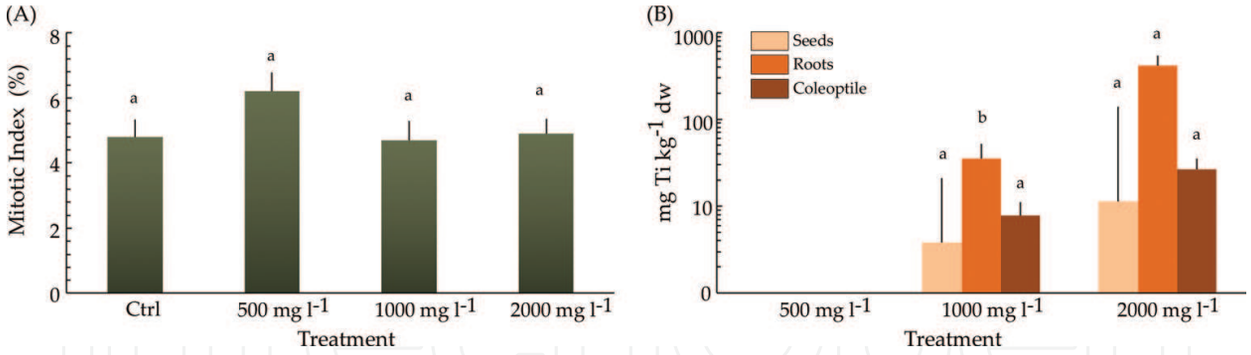

Figure 4. Effects of $n\text{TiO}_2$ on seedlings of *Hordeum vulgare*. (A) Mitotic index (%) (mean ± SE; $n = 3$) observed in root tips of $n\text{TiO}_2$-treated seedlings. (B) Concentration of Ti in seeds, roots, and shoots (mean ± SE; $n = 3$) of $n\text{TiO}_2$ barley-treated seedlings. Different letters indicate statistical difference between treatments at Tukey's test ($p < 0.05$).

3.3. Life cycle study

3.3.1. Plant growth

The data obtained from the phenological observation are shown in **Figure 5**. The barley plants grown in soil spiked with $n\text{TiO}_2$ at 500 and 1000 mg kg^{-1} are in delay with respect to the control barley plants in reaching each physiological maturity; this delay is already appreciable from the second leaf stage. At the end of the physiological maturity, the barley plants were used for measuring the representative parameters of plant growth in particular, the plant height, number of tillers, total leaves area, and grain yield (**Figure 6**). The plant height was not affected in a significant way by the $n\text{TiO}_2$ treatments; however, there is a gradual increment of plant height with the increase of $n\text{TiO}_2$ concentration in the soil. The number of tillers, like the plant height parameter, increases at the increase of $n\text{TiO}_2$ into the soil. Differently from the previous parameter, the average number of tillers of barley plants grown in the soil spiked with $n\text{TiO}_2$ at 2000 mg l^{-1} show almost significant difference from the other treatments. The total leaf area parameter has the same trend of number of tillers parameter, also in this case there is an increase of leaves surface at the increase of $n\text{TiO}_2$ in the soil with a slightly significant difference in the average value obtained for the plants, which were grown in the soil spiked with 1000 mg kg^{-1} of $n\text{TiO}_2$. The last parameter took into account has been the plant yield. This parameter was affected by the treatments in a different way with respect to the other ones; in fact, the control barley plants did not significantly differ from the barley plants treated with 1000 mg kg^{-1} of $n\text{TiO}_2$ except for the barley plants grown in the soil spiked with 500 mg kg^{-1} of $n\text{TiO}_2$ resulted significantly affected.

3.3.2. Spectroscopy analysis

The spiked soil and the barley plant portions were analyzed by ICP-OES and ICP-MS in order to check the total concentration of Ti (**Table 1**). The soils spiked with $n\text{TiO}_2$ at 500 and 1000 mg l^{-1} have a significant difference from the soil without $n\text{TiO}_2$, though the soil spiked with $n\text{TiO}_2$ at 500 mg l^{-1} results slightly different from the control soil. These results confirm that the soil spiking was performed in a correct way. The analyses of the barley plant portions show there

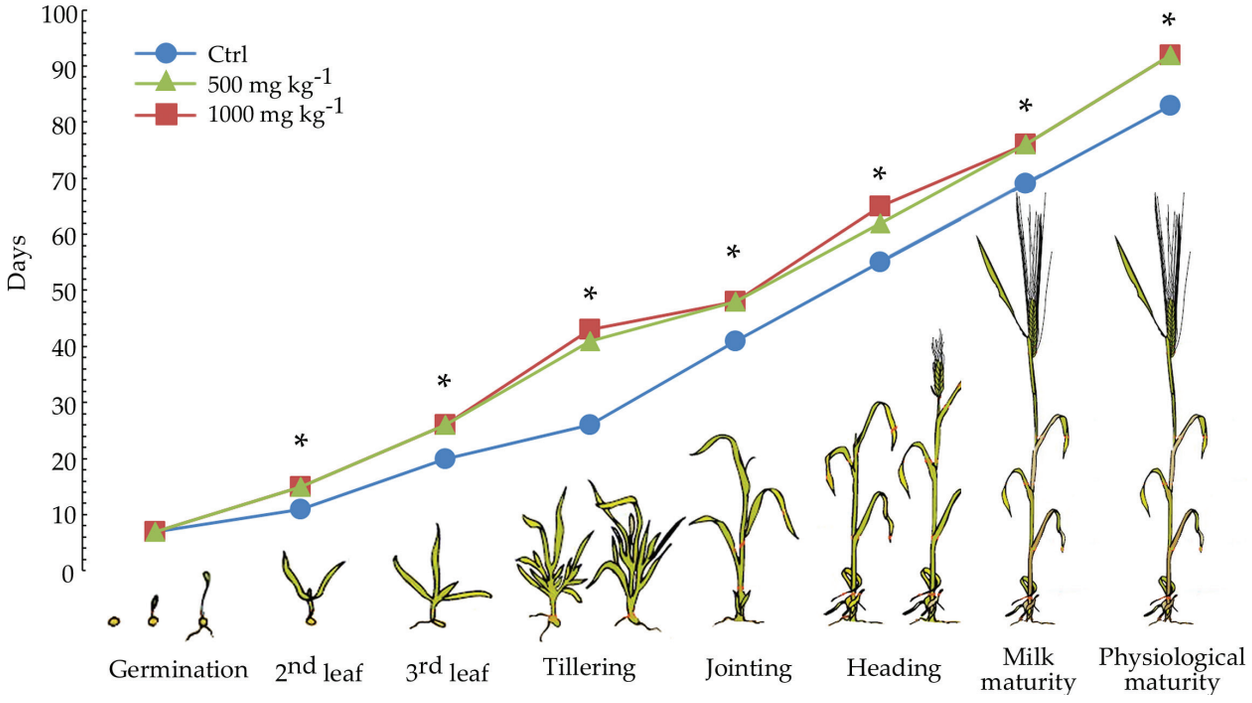

Figure 5. Duration of vegetative and reproductive phenological phases of *Hordeum vulgare* grown in control soil and nTiO$_2$-spiked soil. Asterisk denotes significant differences between control and treated plants ($p \leq 0.05$).

are no significant differences in the Ti concentrations both between the treatments and between treatments and control. The only exception is the total Ti concentration in the stem portion of the plants treated with nTiO$_2$ at 1000 mg kg^{-1} which results slightly different from the same portion of the other plants.

3.3.3. TEM observations

To verify the uptake and subsequent translocations of nTiO$_2$ from roots to aerial plant fractions, ultrastructural analyses on plant leaf tissues were carried out. Rare clusters of nanoparticles were found in leaves sampled from plants grown in soil enriched with the different combinations of nTiO$_2$, at both concentrations (**Figure 7**). nTiO$_2$ were observed in leaf cells and, in particular, in the stroma of the chloroplast and in the vacuoles. Despite the treatment, the chloroplast ultrastructure appeared normal (**Figure 7B**).

3.3.4. Macronutrient and micronutrient concentrations in kernels

The accumulation of macronutrients in barley kernels is shown in **Table 2**. Both N and S concentrations increase at the increment of nTiO$_2$, whereas for Ca there was not the same behavior. Apparently, K, P, and Mg concentrations in kernels did not respond to the treatment. **Table 3** reports the concentrations of micronutrients in kernels. The nTiO$_2$ treatments determined an increase in Fe, Mn, and Zn concentrations in barley kernels, whereas B and Cu concentrations were not influenced by the treatments.

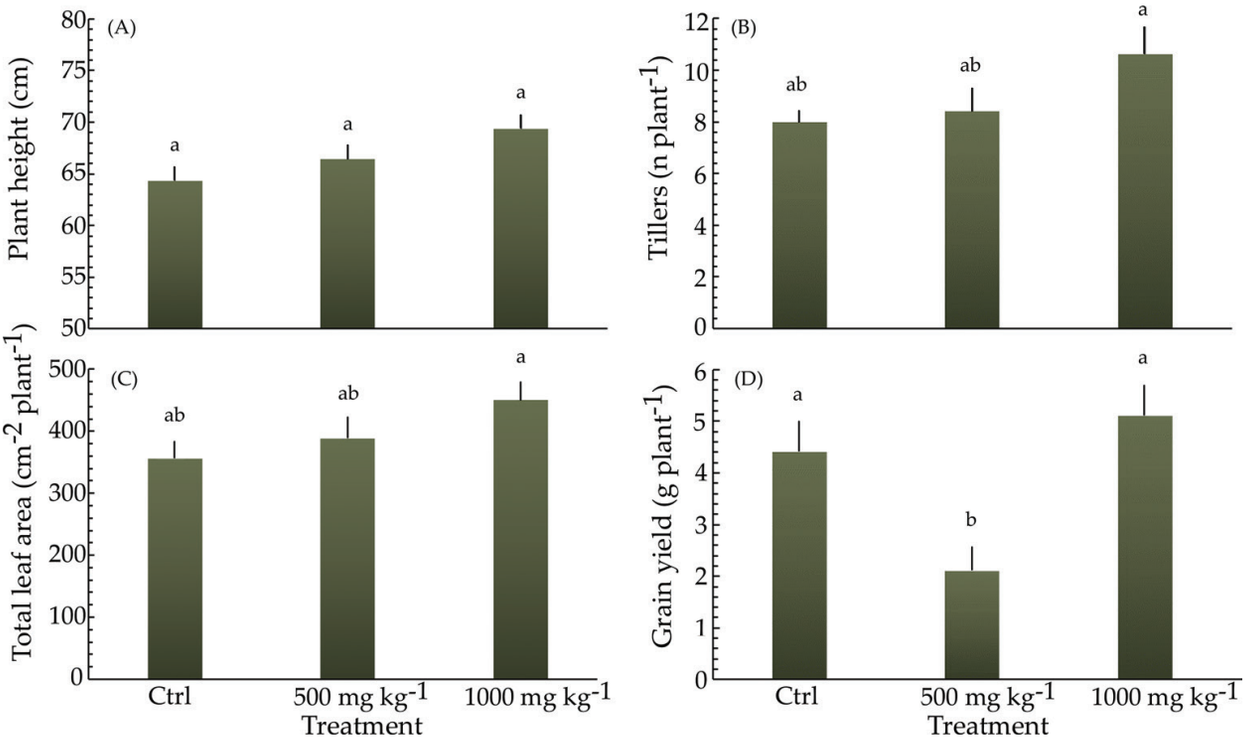

Figure 6. Biometric variables of *Hordeum vulgare* observed in plants grown in control soil and nTiO$_2$-spiked soils. Variables are respectively: (A) plant height, (B) number of tillers per plant, (C) total leaf area per plant, and (D) grain yield per plant. Bars are mean standard error (n = 5). Different letters indicate statistical difference between treatments at Tukey's test (p < 0.05).

Treatment	Soil (mg kg^{-1})	Roots (mg kg^{-1})	Stems (mg kg^{-1})	Leaves (mg kg^{-1})	Spike (μg kg^{-1})
Ctrl	1797 ± 119 b	77 ± 3.19 a	0.26 ± 0.04 ab	1.03 ± 0.06 a	2.19 ± 1.2 a
Ti 500	2153 ± 119 ab	66.7 ± 7.49 a	0.28 ± 0.03 ab	1.39 ± 0.35 a	1.71 ± 0.53 a
Ti 1000	2537 ± 56.3 a	81.7 ± 4.96 a	0.39 ± 0.06 a	0.96 ± 0.09 a	1.39 ± 0.27 a

Values are mean ± SE (n = 5). Same letters indicated no statistical difference between treatments at Tukey's test ($p \leq 0.05$).

Table 1. Ti concentration observed in soil, roots, stems, leaves, kernels of primary and secondary spikes of barley plants grown in control (Ctrl) and nTiO$_2$-spiked soil.

3.3.5. Amino acids in kernels

The effects of nTiO$_2$ treatments on amino acid concentrations in kernels are shown in **Table 4**. Overall, Glu and Pro are the most abundant amino acids in kernels with concentration ranges of 31–43 and 15–21 mg·g^{-1}, respectively. The nTiO$_2$ treatments did not significantly modify concentrations of Ala, Arg, Asp, His, Ser, and Trp. On the opposite, the concentration of Cys, Glu, Gly, Ile, Leu, Lys, Phen, Pro, Tyr, and Val in kernels significantly increased in response to the nTiO$_2$ treatments. In the case of Thr, the response to the treatment was less evident. At last, only in the case of Met contradictory results were recorded.

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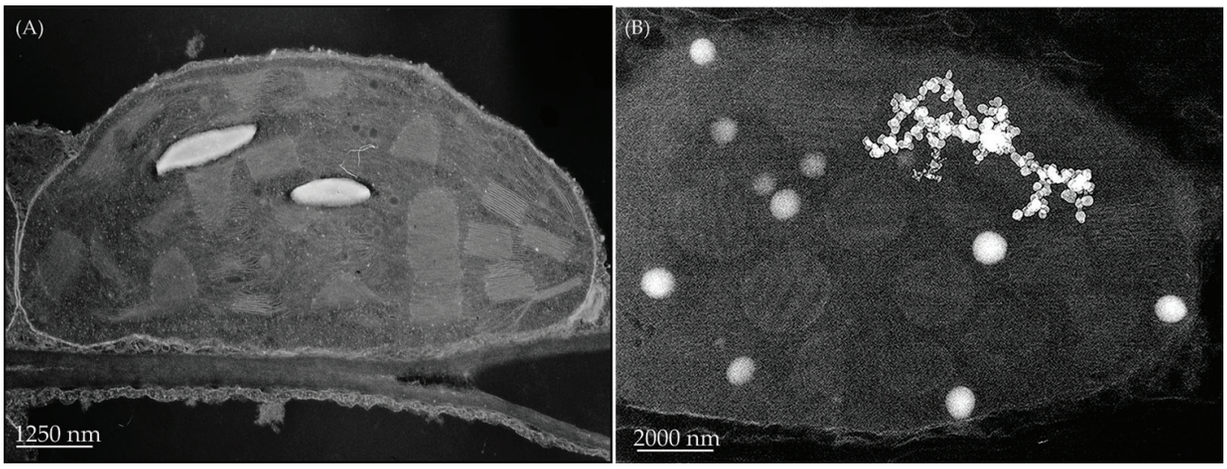

Figure 7. Representative TEM micrograph of leaf tissues of *Hordeum vulgare* plants grown in (A) control soil and (B) nTiO$_2$ 1000 mg kg^{-1}-spiked soils. Clusters of Ti nanoparticles are visible in the stroma of the chloroplasts of nTiO$_2$-treated plants (B).

Element	Ctrl	nTiO$_2$ 500 mg kg^{-1}	nTiO$_2$ 1000 mg kg^{-1}
N (% dw)	2.22 ± 0.08 b	2.62 ± 0.12 a	2.78 ± 0.05 a
K (g kg^{-1})	4.57 ± 0.10 a	4.19 ± 0.16 ab	3.83 ± 0.06 b
P (mg kg^{-1})	4.55 ± 0.33 a	4.87 ± 0.23 a	4.36 ± 0.31 a
Ca (mg kg^{-1})	377 ± 21 b	701 ± 81 a	543 ± 8 ab
Mg (mg kg^{-1})	1881 ± 59 a	1983 ± 124 a	1731 ± 44 a
S (mg kg^{-1})	1814 ± 128 b	2391 ± 47 a	2027 ± 161 b

Values are mean ± SE (n = 5). Same letters indicated no statistical difference between treatments at Tukey's test ($p \leq 0.05$).

Table 2. Nitrogen percentage and concentration of macronutrients in barley kernels at ripening from main shoot grown in control soil (Ctrl) and nTiO$_2$-spiked soil.

Element	Ctrl	nTiO$_2$ 500 mg kg^{-1}	nTiO$_2$ 1000 mg kg^{-1}
B (mg kg^{-1})	8.64 ± 1.02 a	8.01 ± 1.7 a	6.02 ± 1.58 a
Cu (mg kg^{-1})	8.91 ± 1.33 a	7.52 ± 1.16 a	8.24 ± 0.28 a
Fe (mg kg^{-1})	46.4 ± 9.80 b	197 ± 43.7 a	101 ± 27 ab
Mn (mg kg^{-1})	18.8 ± 0.64 b	25.1 ± 1.06 a	21.6 ± 1.23 ab
Zn (mg kg^{-1})	55.7 ± 5.36 b	69.6 ± 2.61 a	59.6 ± 1.34 ab

Values are mean ± SE (n = 5). Same letters indicated no statistical difference between treatments at Tukey's test ($p \leq 0.05$).

Table 3. Concentration of micronutrients in barley kernels at ripening from main shoot grown in control soil (Ctrl) and nTiO$_2$-spiked soil.

Amino acid	Ctrl	$n\text{TiO}_2$ 500 mg kg^{-1}	$n\text{TiO}_2$ 1000 mg kg^{-1}
Alanine (Ala)	5.65 ± 0.51 a	7.35 ± 1.05 a	6.75 ± 0.16 a
Arginine (Arg)	7.55 ± 1.32 a	9.26 ± 0.56 a	9.12 ± 0.83 a
Aspartic acid (Asp)	7.18 ± 0.67 a	8.58 ± 0.65 a	9.09 ± 0.49 a
Cysteine (Cys)	6.85 ± 0.13 b	8.07 ± 0.01 a	8.42 ± 0.36 a
Glutamic acid (Glu)	31.2 ± 3.56 b	40.7 ± 3.73 a	43 ± 1.83 a
Glycine (Gly)	5.98 ± 0.45 b	7.74 ± 0.29 a	8.01 ± 0.42 a
Histidine (His)	3.14 ± 0.51 a	3.66 ± 0.18 a	3.89 ± 0.18 a
Isoleucine (Ile)	5.29 ± 0.47 b	6.42 ± 0.36 a	6.77 ± 0.25 a
Leucine (Leu)	9.4 ± 0.74 b	11.2 ± 0.81 a	11.7 ± 0.42 a
Lysine (Lys)	3.67 ± 0.31 b	5.85 ± 0.33 a	5.98 ± 0.45 a
Methionine (Met)	2.39 ± 0.13 b	3.08 ± 0.01 a	3 ± 0.20 b
Phenylalanine (Phe)	7.48 ± 0.94 b	9.12 ± 0.65 a	9.37 ± 0.45 a
Proline (Pro)	14.8 ± 1.68 b	20.4 ± 3.04 a	21.4 ± 1.41 a
Serine (Ser)	5.84 ± 0.54 a	6.78 ± 0.36 a	6.84 ± 0.18 a
Threonine (Thr)	4.61 ± 0.31 a	5.11 ± 0.36 ab	5.35 ± 0.18 ab
Tryptophan (Trp)	1.15 ± 0.67 a	0.53 ± 0.01 a	0.75 ± 0.18 a
Tyrosine (Tyr)	3.36 ± 0.42 b	4.34 ± 0.20 a	4.22 ± 0.36 a
Valine (Val)	7.04 ± 0.49 b	8.29 ± 0.65 a	8.68 ± 0.40 a

Table 4. Amino acid ($mg \cdot g^{-1}$) concentration in barley kernels at ripening from main shoot grown in soil spiked with none (Control), 500 mg $n\text{TiO}_2 \cdot kg^{-1}$, and 1000 mg $n\text{TiO}_2 \cdot kg^{-1}$.

4. Discussion

The $n\text{TiO}_2$ suspensions did not affect germination of *H. vulgare*. Our results are in agreement with the observations carried out, respectively, on rice [19], lettuce, radish, and cucumber [20], tomato [21], and pea [22]. According to Ref. [11], we demonstrated that $n\text{TiO}_2$ treatment did not affect root elongation of seedlings. Other authors published opposite results. In fact, Mushtaq [23] showed an inhibitory effect of $n\text{TiO}_2$ on root elongation in cucumber, whereas Fan et al. [22] verified decrease in the number of secondary lateral roots in pea. The $n\text{TiO}_2$ treatments did not influence the mitotic index. That is in contrast with Moreno-Olivas et al. [24] which observed a $n\text{TiO}_2$-induced genotoxicity in hydroponically cultivated zucchini. Although the size of $n\text{TiO}_2$ used in that experiment is comparable to ours, those experiments were carried out in different conditions than ours. This can result in different experimental conditions, with particular regard to the $n\text{TiO}_2$ traits (e.g., different grade of agglomeration due to different z-average size and zeta potential). On the other hand, the results obtained

by ICP-OES analyses seem to indicate a $n\text{TiO}_2$ uptake by root tissue and a subsequent translocation in the other seedling tissues. This result could be an indication of a real uptake and translocation of $n\text{TiO}_2$. A second hypothesis is that, despite the use of appropriate analytical protocols, the analysis may have been disturbed by sample contamination. In that case, the element concentration in the plant tissues could be significantly overestimated due to a fraction of metal simply adsorbed onto the external sample surface.

With regard to the effects along the entire life cycle, the response of plant phenology was in accordance with previous studies [25, 26]. In fact, the barley plants treated with $n\text{TiO}_2$ result to have a longer vegetative phase. During this phase, the plants keep growing and the leaves continue their photosynthetic activity and consequently the production of photosynthates [27]. Taking into account such evidences, a higher biomass production and grain yield in treated plants respect the control ones it is expected. The analyses of biometric parameters confirm in part the expected results. Except for the plant height and grain yield per plants the other parameters result positively affected. Our results confirm other experimental evidences. In particular, studies carried out on *Spinacia oleracea* have demonstrated that $n\text{TiO}_2$ promotes plant photosynthesis increasing light absorbance and transformation of light energy and enhancing Rubisco activity [28, 29]. The positive effects of $n\text{TiO}_2$ treatments were evidenced also at the grain level. The grains obtained from treated plants result to have a higher content of macro (Na and Ca) and micronutrients (Fe, Mn, and Zn); moreover, a positive effect of $n\text{TiO}_2$ treatment was also observed for several amino acids. The increase of macro/micronutrient and amino acid concentrations in kernels could be an effect related with the longest vegetative phase caused by the $n\text{TiO}_2$ treatments. In order to find the relationship between the effects and treatment, the material obtained at the end of the experiment was analyzed by ICP-OES and observed by TEM. The ICP-OES analyses and the TEM observations were carried out in order to know if the $n\text{TiO}_2$ can enter into plant tissues and subsequently cause the observed effects on plants. The ICP-OES results did not put on evidence an effective uptake of titanium by the plants, but the TEM observations show the presence of $n\text{TiO}_2$ in the stroma of the chloroplast and in the vacuoles of leaf cells. This discrepancy in results could be related with the agglomeration tendency of $n\text{TiO}_2$ in water, previously evidenced by $n\text{TiO}_2$ characterization analyses. The agglomeration makes the $n\text{TiO}_2$ less available because their increased dimension makes difficult the passage of them through the cell wall, this means only the $n\text{TiO}_2$ with the smallest size can pass this plant barrier and consequently small amount of titanium could be uptaken.

5. Conclusions

The amount of products containing nanoparticles will increase in the future years; this will bring an increase of their presence in the environment. In the last years, the majority of literature was focused to investigate the potential negative impact of this new kind of material on human, animals, and plants, but in our study, we put on evidence the potential beneficial effects. At first, we demonstrate the absence of negative impact during the early development

stages of barley plants; in fact, each $n\text{TiO}_2$ concentration did not affect the germination percentage and root elongation, except for the lowest concentration ($n\text{TiO}_2$ 500 mg kg^{-1}) which significantly affects in a negative way the last parameter. The analysis focus moves to evaluation of the possible effects at genetic level; for this purpose, the mitotic index was analyzed. The results also show, in this case, the absence of an effect for this parameter. The AFM and DLS analyses give information about the tendency of $n\text{TiO}_2$ to form big agglomerate once dissolved in MilliQ water, this makes the $n\text{TiO}_2$ less available for the seeds/seedlings, and the absence of effects could be related to the incapacity of $n\text{TiO}_2$ to cross the cell wall. However, the ICP-OES and ICP-MS analyses demonstrate the capacity of seeds/seedlings to uptake the titanium, then the absence of effects in the early developmental stages is not due to the absence of titanium in the plant tissues but to this unharmful effect. The experiment set up to evaluate the possible effect of $n\text{TiO}_2$ along the entire barley life cycle demonstrates the positive dose-response effect on vegetative growth, and this has a direct effect on the composition and nutritional value of barley grains.

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References

[1] Handford CE, Dean M, Henchion M, Spence M, Elliott CT, Campbell K. Implication of nanotechnology for the agri-food industry: Opportunities, benefits and risks. Trends in Food Science & Technology. 2014;**40**:226-241. DOI: http://dx.doi.org/10.1016/j.tifs.2014.09.007

[2] Doyle ME. Nanotechnology: A Brief Literature Review. Food. Madison, WI: University of Wisconsin-Madison; 2006. p. 10

[3] Mousavi SR, Rezaei M. Nanotechnology in agriculture. Journal of Applied Environmental and Biological Sciences. 2011;**1**(10):414-419

[4] Capaldi Arruda SC, Diniz Silva AL, Galazzi RM, Azevedo RA, Zezzi Arruda MA. Nanoparticles applied to plant science: A review. Talanta. 2015;**131**:693-705. DOI: http://dx.doi.org/10.1016/j.talanta.2014.08.050

[5] Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL. Interaction of nanoparticles with edible plants and their possible implications in the food chain. Journal of Agricultural and Food Chemistry. 2011;**59**(8):3485-3498. DOI: http://dx.doi.org/10.1021/jf104517j

[6] Zheng L, Hong F, Lu S, Liu C. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research. 2005;**104**(1):83-91

[7] Hong F, Zhou J, Liu C, Yang F, Wu C, Zheng L, et al. Effect of nano-TiO_2 on photochemical reaction of chloroplasts of spinach. Biological Trace Element Research. 2005; **105**(1-3):269-279. DOI: http://doi:10.1385/BTER:105:1-3:269

[8] Yang F, Liu C, Gao F, Su M, Wu X, Zheng L, et al. The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. Biological Trace Element. 2007;**119**(1):77-88. DOI: http://doi:10.1007/s12011-007-0046-4

[9] Gao F, Liu C, Qu C, Zheng L, Yang F, Su M, et al. Was improvement of spinach growth by nano-TiO_2 treatment related to the changes of Rubisco activase? BioMetals. 2008;**21**(2):211-217. DOI: http://doi:10.1007/s10534-007-9110-y

[10] Dehkourdi EH, Mosavi M. Effect of anatase nanoparticles (TiO_2) on parsley seed germination (*Petroselinum crispum*) in vitro. Biological Trace Element Research. 2013;**155** (2):283-286. DOI: http://doi:10.1007/s12011-013-9788-3

[11] Feizi H, Amirmoradi S, Abdollahi F, Pour SJ. Comparative effects of nanosized and bulk titanium dioxide concentrations on medicinal plant *Salvia officinalis* L. Annual Review & Research in Biology. 2013;**3**(4):814-824

[12] Qi M, Liu Y, Li T. Nano-TiO_2 improve the photosynthesis of tomato leaves under mild heat stress. Biological Trace Element Research. 2013;**156**(1-3):323-328. DOI: http://doi:10.1007/s12011-013-9833-2

[13] Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW. Applications of nanomaterials in agricultural production and crop protection: A review. Crop Protection. 2012;**35**:64-70. DOI: http://dx.doi.org/10.1016/j.cropro.2012.01.007

[14] Marchiol L, Mattiello A, Pošćić F, Fellet G, Zavalloni C, Carlino E, et al. Changes in physiological and agronomical parameters of barley (*Hordeum vulgare*) exposed to cerium and titanium dioxide nanoparticles. International Journal of Environmental Research and Public Health. 2016;**13**(3):332. DOI: http://doi:10.3390/ijerph13030332

[15] Schneider CA, Rasband WS, Eliceiri K. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012;**9**(7):671-675. DOI: http://doi:10.1038/nmeth.2089

[16] Priester JH, Ge Y, Mielke RE, Horst AM, Moritz SC, Espinosa K, et al. Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. Proceedings of the National Academy of Sciences. 2012;**109**(37):14734-14735. DOI: http://doi: 10.1073/pnas.1205431109

[17] Zadoks JC, Chang TT, Konzak CF. A decimal code for the growth stages of cereals. Weed Research. 1974;**14**(6):415-421. DOI: http://doi:10.1111/j.1365-3180.1974.tb01084.x

[18] Pošćić F, Mattiello A, Fellet G, Miceli F, Marchiol L. Effects of cerium and titanium oxide nanoparticles in soil on the nutrient composition of barley (*Hordeum vulgare* L.) kernels. International Journal of Environmental Research and Public Health. 2016;**13**(6):577. DOI: http://doi:10.3390/ijerph13060577

[19] Boonyanitipong B, Kositsup B, Kumar P, Baruah S, Dutta J. Toxicity of ZnO and TiO_2 nanoparticles on germinating rice seed *Oryza sativa* L. International Journal of Bioscience, Biochemistry, and Bioinformatics. 2011;**1**:282-285. DOI: http://doi:10.7763/IJBBB.2011.V1.53

[20] Wu SG, Huang L, Head J, Chen DR, Kong IC, Tang YJ. Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals. Petroleum & Environmental Biotechnology. 2012;**3**(4):126. DOI: http://doi:10.4172/2157-7463.1000126

[21] Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J, et al. Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicology and Environmental Safety. 2013;**93**:60-67. DOI: http://doi:10.1016/j.ecoenv.2013.03.033

[22] Fan R, Huang YC, Grusak MA, Huang CP, Sherrier DJ. Effects of nano-TiO_2 on the agronomically-relevant Rhizobium-legume symbiosis. Science of the Total Environment. 2014;**497-498**:503-512. DOI: http://doi:10.1016/j.scitotenv.2013.07.032

[23] Mushtaq YK. Effect of nanoscale Fe_3O_4, TiO_2 and carbon particles on cucumber seed germination. Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering. 2011;**46**:1732-1735. DOI: http://doi:10.1080/10934529.2011.633403

[24] Moreno-Olivas F, Gant VU Jr, Johnson KL, Peralta-Videa JR, Gardea-Torresdey JL. Random amplified polymorphic DNA reveals that TiO_2 nanoparticles are genotoxic to *Cucurbita pepo*. Journal of Zhejiang University SCIENCE A. 2014;**15**:618-623. DOI: http://doi:10.1631/jzus.A1400159

[25] Rico CM, Barrios AC, Tan W, Rubenecia R, Lee SC, Varela-Ramirez A, et al. Physiological and biochemical response of soil-grown barley (*Hordeum vulgare* L.) to cerium oxide nanoparticles. Environmental Science and Pollution Research. 2015;**22**(14):10551-10558. DOI: http://doi:10.1007/s11356-015-4243-y

[26] Yoon SJ, Kwaka JI, Lee WM, Holden PA, An YJ. Zinc oxide nanoparticles delay soybean development: A standard soil microcosm study. Ecotoxicology and Environmental Safety. 2013;**100**(1):131-137. DOI: http://dx.doi.org/10.1016/j.ecoenv.2013.10.014

[27] Dofing SM. Phenological development–yield relationships in spring barley in a subarctic environment. Canadian Journal of Plant Science. 1995;**75**(1):93-97. DOI: doi:10.4141/cjps95-015

[28] Yang F, Hong F, You W, Liu C, Gao F, Wu C, et al. Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. Biological Trace Element Research. 2006;**110**(2):179-190. DOI: http://doi:10.1385/BTER:110:2:179

[29] Gao F, Hong F, Liu C, Zheng L, Su M, Wu X, et al. Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach. Biological Trace Element Research. 2006;**111**(1):239-253. DOI: http://doi:10.1385/BTER:111:1:239

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