C. R. Biologies xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Physiology/Physiologie

Effects of nanosized titanium dioxide on the photosynthetic metabolism of fenugreek (*Trigonella foenum-graecum* L.)

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ARTICLE INFO

Article history:
Received 6 July 2017
Accepted after revision 16 September 2017
Available online xxx

Keywords:
Trigonella foenum graecum L.
TiO_2NPs
Plant biomass
Photosynthetic pigments
Anthocyanins
Flavonoids
Phenols
Oxidative stress

ABSTRACT

The potential toxicity of nanoparticles in plants is scarce and contradictory. Despite the diversity of research efforts, a detailed explanation of the TiO_2NPS effects in plant photosynthesis is still missing. The present work gives a new approach to examine the impact of the TiO_2NPs on crop production (development and photosynthesis) and plant protection (tolerance and defense systems) in fenugreek (*Trigonella foenum graecum* L.). Seedlings were assessed in greenhouse trials to estimate the influence of TiO_2NPs on physiological characters for 16 days. They were treated with TiO_2NPs at a size less than 20 nm. The results revealed that there were no significant effects on seedlings growth and biomass of stem, but a decrease in the fresh weight of leaves after TiO_2NPs treatment. Plants treated with 100 $mg{\cdot}L^{-1}$ of TiO_2NPs presented a reduction and chlorosis in leaf area due to a significant decrease in the chlorophyll *a* and *b* contents. The highest value of the photosynthetic pigments was recorded at 50 $mg{\cdot}L^{-1}$ of TiO_2NPs. However, the treatment with 100 $mg{\cdot}L^{-1}$ of TiO_2NPs caused a decrease in the levels of chlorophyll *a*, *b* and of carotenoids. Both doses of TiO_2NPs induced an accumulation of anthocyanins compared to the control after 16 days of seedling development. A nano-stress significantly decreased the flavonoids level, but increased that of polyphenols compared to control after 16 days of exposure. The decrease in the translocation ratio of flavonoids suggests that many of them contain an enediol group, which suggests that they may act as bidentate ligands for anatase TiO_2NPs. Accordingly, nano-stressed leaves exhibited significantly enhanced GPOX, CAT and APX activity levels. On the contrary, GPOX and CAT activities were reduced substantially in stems treated with 100 $mg{\cdot}L^{-1}$ TiO_2NPs. The accumulation of MDA was found to be higher in stems than in leaves. This could be explained by the accumulation of nanoparticles in different organs; it could be that the stems are the favored targets of nanoparticles. These results underline the necessity for a deeper estimation of nanoparticle ecotoxicity and particularly concerning their interaction with plants.

1. Introduction

In the last few years, there has been a growing interest in nanomaterial production. Quite recently, considerable attention has been paid to bionanotoxicology due to possible effects and interactions with living organisms

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http://dx.doi.org/10.1016/j.crvi.2017.09.004
1631-0691/

[1]. With a size range between 1 and 100 nm, nanoparticles constitute a recently developed technology, largely applied in different fields, such as cosmetics, skin care products, particularly in sun blocks and toothpastes, antibacterial, air cleaning products, and for decomposing organic wastewater treatment, due to their properties, involving high stability as well as anticorrosion and photocatalyst activity [2,3]. For several years, great efforts have been paid to study the types of nanomaterials. Recent researches focus on five groups of NPs, which are carbon nanoparticles, metal oxides, quantum dots, zero value metals, and nanopolymers. Current research on heterogeneous catalysis for catalytic support of a wide variety of metals is focused on titanium dioxide and zinc oxide [4]. Previous studies prove that titanium dioxide nanoparticles (TiO_2NPs) are insoluble in water and are thermally stable. They are obtained from minerals such as anatase, rutile, or brookite.

Many researches on the production and use of nanoparticle-based pesticides and compost have been done. Great effort has been devoted to the study of the possible penetration of the nanoparticles into the food chain through plant uptake and their bioaccumulation [5,6]. In the literature, several hypotheses have been proposed to explain phytonanotoxicology. To solve this issue, many researchers have proposed various methods to study plant toxicity. Research on TiO_2NP effects on the crops shows a variety of approaches depending on nanoparticles concentration, size, reactivity, chemical structure, surface coating, application, experimental methods, and plant species [7,8].

Previous research has shown that leaf area, stem and root lengths, as well as stem and root weights are morphological indicators of plant health. Antisari et al. [9] argue that the concentrations of 10 and 100 ppm of TiO_2NPs at a size of 20 nm enhanced the shoots and roots fresh weights of wheat, whereas concentrations lower than 100 ppm of TiO_2NPs reduced them in a dose-dependent manner. Asli and Neumann [10] have shown that 30 and 1000 mg·L^{-1} of 30-nm-sized TiO_2NPs induced leaf growth in *Zea mays*. However, Fan et al. [11] showed that 35-nm-sized TiO_2NPs reduced the number of lateral roots in pea. In addition, Jaberzadeh et al. [12] claimed that TiO_2NPs enhanced wheat plant growth and yield.

Besides, on a physiological/biochemical basis, Asli and Neumann [10] proved the negative effect of 30 and 1000 mg·L^{-1} of TiO_2NPs in *Zea mays* by monitoring the rate of transpiration. Jacob et al. [13] and Servin et al. [14] have demonstrated that TiO_2NPs caused a decrease in the chlorophyll content in cucumber and *Phaseolus vulgaris* leaves. Lei et al. [15] have also found that the TiO_2NP treatment caused a decrease in the hydrogen peroxide, superoxide radicals, and malonyldialdehyde contents in the chloroplasts of spinach seedlings due to the activation of superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase. Similarly, the results obtained by Jacob et al. [13] suggest that 10 and 30 ppm of TiO_2NPs enhanced the antioxidant enzyme activities in *Phaseolus vulgaris*. In Wang et al. [16], it was shown that 20 μM of TiO_2NPs with 2.8 nm caused the excretion of microtubules in *Arabidopsis thaliana*. As reported by Yang et al. [17] and Mishra et al. [18], TiO_2NPs controlled nitrogen metabolism by enhancing enzyme activities and by converting inorganic nitrogen to organic nitrogen into the form of protein and chlorophyll. Another interesting approach to this issue has been proposed by Wang et al. [16], who reported that the effects of TiO_2NPs were also on a genetic and molecular basis. Indeed, TiO_2NP treatment increased the tubulin monomers that eventually influence the proteosome system in *Arabdiopsis thaliana*. Castiglione et al. [2] studied the fragmentation of chromosomes in *Vicia narbonensis* and showed that TiO_2NPs caused chromosomal aberrations.

Gao et al. [19] developed a novel sensor using nanoparticles as biostimulants. In this work and in related references, it was observed that TiO_2NPs ameliorate light absorbance and conversion from light energy to electrical and chemical energy, and also induced carbon dioxide assimilation by activating rubisco carboxylation, prevented chloroplasts from aging [17,20,21] and induced the expression of rubisco activase genes [22]. Recently, several authors [23–25] have proposed a regulation of photosynthetic rate, water conductance, transpiration rate and growth in plants by TiO_2NPs.

However, to the author's knowledge, few publications can be found in the literature that address the issue of the biodisponibility, uptake and transport of TiO_2NPs in plants and the mechanism of toxicity on growth and yield. The major drawback of this approach is plant production directly related to the process of photosynthesis. However, most of the previous studies do not consider indirectly the relation of growth and yield with the defense system under TiO_2NPs. Siddiqui et al. [7] analyses and compares various aspects of the effects of TiO_2NPs in plants. Nevertheless, there are still some interesting and relevant problems to be addressed. Transformation or complexation is a critical factor that affects the fate and toxicity of NPs in living organisms.

Although several studies have indicated differences in the responses of plants to TiO_2NPs, little attention has been paid to Fenugreek (*Trigonella foenum-graecum* L.). It is an annual plant belonging to the family of Fabaceae (Papilionaceae) within the order Leguminosae (Leguminales); it is one of the oldest known medicinal plants. Also, it was cultivated especially in India, in Middle Eastern countries, and largely in the Mediterranean basin, including Tunisia. Because of the presence of important phytochemicals elements such as galactomamnan, disogenin and 4-hydroxyisoleucine, Fenugreek has been proven to be essential for the pharmaceutical, nutraceutical, and functional food industries. It is a phytochemically-rich chemurgic plant with potential agronomic, pharmaceutical, and nutraceutical properties [26]. In addition to its therapeutic effects, it is known to accumulate large quantities of heavy metals [27]. After fenugreek germination, the seedlings produce the first leaf, which is usually simple, since there is still no noticeable epicotyl as the first trifoliate leaf is formed after a further 5–8 days [28]. The paper presents a new approach to examine the impact of TiO_2NPs on crop production (development and photosynthesis) and plant protection (tolerance and defense systems). Considering the mentioned impact of TiO_2NPs on physiological processes, the hypothesis of this study is