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THE EFFECT OF TiO_2 AND ZnO_2 NANOPARTICLES UPON SOME BIOMETRICAL CHARACTERISTICS IN SOYBEAN (*Glycine max* L. Merril) *IN VITRO* CULTURES

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Abstract

The aim of this study was to investigate the effects of two different nanoparticles TiO_2 (TiO_2 NPs) and ZnO_2 (ZnO_2 NPs) on the *in vitro* culture of soybean plants *(cv. Felix.). The TiO_2 and ZnO_2 NPs concentrations used for soybean tissue culture were 10, 100 and 1000 mg/l added to MS medium which contained no plant growth regulators. After four weeks, average height of the plants, average length of the main roots, secondary roots and fresh weight of the plantlets were measured. The results show that relatively low concentrations of TiO_2 NPs (10 and 100 mg/l) when added to the culture medium didn't have deleterious effects, but stimulated the growth and development of soybean plants. When higher concentrations (1000 mg/l) of TiO_2 NPs were added the plant growth was inhibited. The average fresh weight of a plantlet was 2200,14±51,56 mg on the medium without TiO_2 NPs and 2270,03±39,78 mg at the concentration of 100 mg/l TiO_2 NPs added. At the highest concentration of TiO_2 NPs added the average weight of the plant decreased significantly to 1616,86 ± 68,09 mg. The addition of ZnO_2 NPs to the culture media at higher concentrations than 10 mg/l showed a clear inhibitory effect on plant growth. Therefore, our results suggest that ZnO_2 NPs had a greater inhibitory effect on soybean plant growth and development than the TiO_2 NPs.*

Key words*: micropropagation, nanoparticles, titanium, zinc.*

INTRODUCTION

Nanoparticles of TiO_2 and ZnO_2 are used already on a large scale in many industries such as food, pharmaceutical, cosmetics, plastics, paper and it is expected that in the near future to become a possible source of danger of toxicity for our environment since its quantity is increasing (Coman et al., 2019; Javed et al., 2017; Zafar et al., 2016; Demir et al., 2014; Safavi, 2014; Prasad et al., 2012; Klančnik et al., 2011).

Previous reports highlight the positive but also the negative effects of ZnO_2 nanoparticles on different agricultural crops; their effects being influenced by their concentration (Javed, 2017; Zafar et al., 2016).

Other studies show that also the presence of TiO_2 nanoparticles can have both positive and negative effects on different plants' growth such as rice, peanut, black mustard, soybean, onion, sugarleaf etc. (Chutipaijit & Sutjaritvorakul, 2017; Laware & Raskar, 2014; Safavi, 2014). Among these plants, soybean (*Glycine max*), which is considered one of the most important agricultural crops plants in terms of seed protein and oil contents, has proven to be a perfect model for nanoparticle accumulation studies due to its high biomass production and ease of cultivation (Coman et al., 2019). However, to the best of our knowledge, there is no any report published about the effect of TiO_2 and ZnO_2 on *in vitro* culture of soybean plants. Therefore, the aim of this research was to investigate the effect of TiO_2 and ZnO_2 nanoparticles in different concentrations ranging from 10 to 1000 mg/l on the development of soybean cv. Felix grown in vitro. We strongly believe that the experimental

results would considerably contribute to the enrichment of knowledge regarding the interaction of TiO_2 and ZnO_2 nanoparticles with plant mechanisms.

MATERIALS AND METHODS

Characterization of TiO_2 and ZnO_2 nanoparticles

The nanoparticles used in this experiment were purchased from Sigma-Aldrich and Merck: TiO_2 (TiO_2 NPs): code 700347-25G, Lot # MCBT6314V Titanium (IV) oxide, mixture of rutile and anatase nanoparticles, < 150 nm particle size (volume distribution, DLS), dispersion, 40 wt. % in H_2O, 99.5% trace metals basis and ZnO_2 (ZnO_2 NPs) code 721077 - 100G, Lot # MKCC4480 Zinc oxide, dispersion nanoparticles, < 100 nm particle size (TEM), ≤ 40 nm avg. part. size (APS), 20 wt. % in H_2O.

Media preparation with TiO_2 and ZnO_2 nanoparticles

To investigate the toxicity of effect of Ti NPs and Z NPs plain Murashige and Skoog 1962 (MS) agar media was used (Murashige and Skoog, 1962) with no plant growth regulators added (Duchefa Biochemie B.V. Olanda, M0222, Murashige and Skoog 1962 (MS) medium including vitamins, original concentration 4405.19 mg/l). Regular sugar was added as carbon source and plant agar was used to solidify the culture media (4 g/l plant agar, Duchefa Biochemie B.V. Olanda, cod P1001**,** Plant Agar, Cas number 9002-18-0, Gel strength min. 1100 g/cm^2, Crude ash < 3%, Ash, acid insoluble < 0.5%). The pH of the media was adjusted to 5,8 before the plant agar was added as solidifying agent. The medium was dispensed (8 ml) in glass tubes of 140/25 mm. The media was autoclaved at 121ºC for 20 minutes, 1 atm. The TiO_2 NPs and ZnO_2 NPs, in concentrations of 0, 10, 100 and 1000 mg/l were added to the culture medium before autoclavation.

In vitro Culture

Felix soybean cultivar was chosen and used as plant material to carry out this experiment. This variety was crated at the Agricultural Research and Development Station Turda-Cluj as a result of the cross of Maple presto x Merit. This soybean variety develops a compact bush, erect port, and semi-definite growth. It is a high-grade variety with an average height of 94 cm. The average mass of 1000 soybean is 178 grams. The average vegetation period is 122 days (Mureşanu et al., 2010). To initiate the *in vitro* culture, the seeds of 'Felix' soybean were washed through running tap water for 10 minutes and then rinsed with double-distilled water containing one drop of Tween using a magnetic stirrer. Then, the seeds were rinsed again using double distilled water to eliminate the rest of the Tween solution. In the next step, in aseptic conditions the seeds were treated with ACE solution (20%) for 20 minutes and then rinsed with double-distilled water several times thoroughly. One seed was inoculated in each glass tube (Figure 1 a, b). The culture vessels were kept in growth chamber at 36 μmol m^{-2} s^{-1} light intensity provided by white fluorescent tubes (Philips, 36 W), at 23 ± 2°C and 50 ± 5% humidity.

Data analysis

To investigate the effects of ZnO and TiO2 NPs seed germination and plant characters, 20 glass tubes of each treatment were inoculated with 1 seed/tube in three repetitions. To analyse the explant responses, 10 plantlets/treatment were randomly selected for measurements. After four weeks of culture various biometrical measurements were made as follow: plantlets' height, length of the main and secondary roots, fresh weight of the plantlets. The results are presented as mean values with standard error. The means were further analysed using ANOVA and Tukey's HSD test ($p < 0.05$) to determine the differences among the means.

RESULTS AND DISCUSSIONS

Effect of TiO_2 NPs on soybean in vitro regeneration

The presence of TiO_2 NPs in the culture media in low concentration (10 mg/l) did not affect negatively but stimulated plantlet growth. The highest plants were developed on the culture media with 10 mg/l TiO_2 NPs concentration reaching 16.79 ± 0.40 cm. The increase of NP's concentration inhibited plant growth; at 1000 mg/l Ti NPs the average plant height recorded was 14.61 ± 0.34 cm. Regarding the maximum average length of primary and secondary roots, there were no statistically significant differences between the control and the concentration of 10 NPs, while at 1000 mg/l NPs concentration root

length decreased significantly by 47.03% (7.74 ± 0.27 cm) for both primary and secondary roots (Figure 2). Furthermore, the number of secondary roots was significantly lower at 1000 mg/l TiO_2 NPs in comparison with other concentrations (Figure 3). The average fresh weight of the plantlets on the media without NPs was 2200.14 ± 51.56 mg/plantlet and increased to 2270.03 ± 39.78 mg/plantlet at the concentration of 100 mg/l TiO_2 NPs. At 1000 mg/l TiO_2 NPs decreased by 26.52% 1616.86 ± 68.09 mg/plantlet (Figure 4).

Similar growing patterns were observed in *Oryza sativa* (Chutipaijit & Sutjaritvorakul, 2017) when different TiO_2 NPs concentrations were added to the culture media (0, 5, 10, 15, 20, 25 mg). The results show, that up to the concentration of 20 mg the nanomaterials stimulated plant regeneration. Thus, the culture medium supplemented with 20 mg l^{-1} TiO_2 nanoparticles provided the highest frequency (67%) of plant regeneration from mature seeds of indica rice cv. RD49 while higher concentrations inhibited plant growth. In addition, the results also suggest that optimum concentration of TiO_2 nanoparticles has significantly enhanced the percentages of callus induction and plant regeneration of *Oryza sativa* cultivars (Suphanburi1 and Suphanburi 90 cultivars). This research highlights also the efficiency of the culture medium supplemented with TiO_2 nanoparticles for in vitro micropropagation from mature seeds of Suphanburi1 and Suphanburi90 cultivars. Culture medium supplemented with 50 mg l^{-1} and 40 mg L^{-1} TiO_2 NPs were selected as the best media composition for callus induction and plant regeneration (Chutipaijit & Sutjaritvorakul, 2018).

Figure 1. *In vitro* culture of *Glycine max* L. 'Felix' with different TiO_2 and ZnO_2 NPs concentrations: a,b - *in vitro* culture initiation; c - culture media with 0, 10, 100 and 1000 mg/l ZnO NPs; d - *in vitro* culture of soybean with 0, 10, 100 and 1000 mg/l NPs of TiO_2.

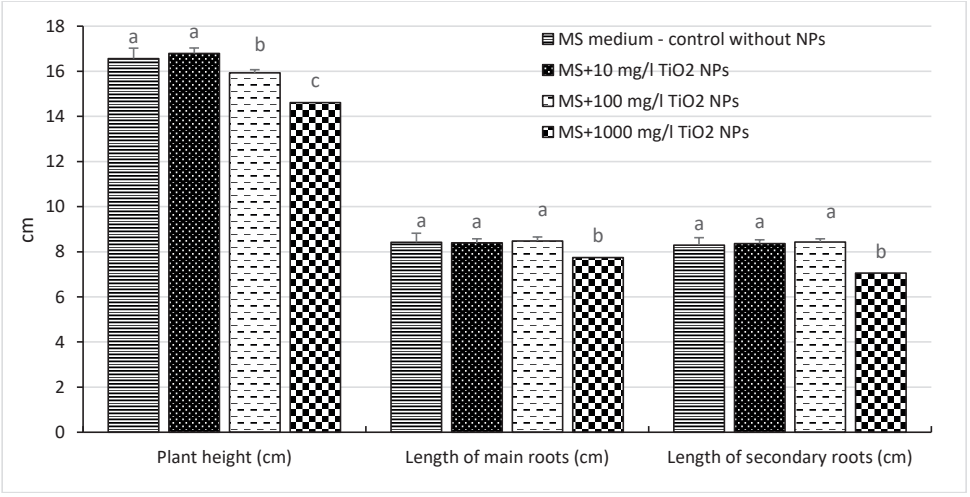

Figure 2. Effect of TiO_2 NPs on *in vitro* cultured *Glycine max* L. 'Felix' plantlet length, root length (primary and secondary) The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

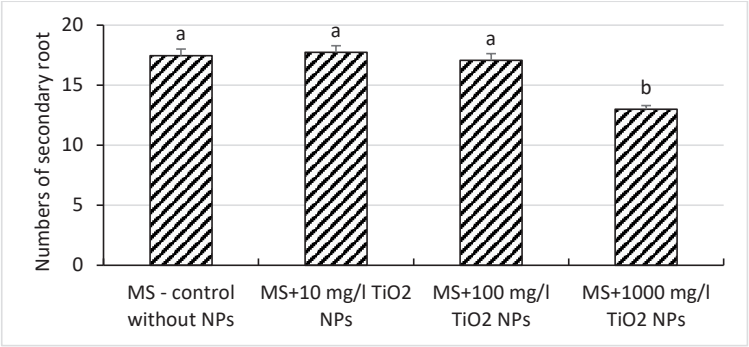

Figure 3. Effect of TiO_2 NPs on *in vitro* cultured *Glycine max* L. 'Felix' number of primary and secondary roots. The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

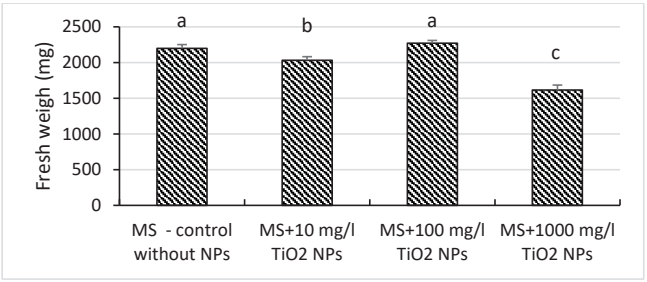

Figure 4. Effect of TiO_2 NPs on *in vitro* cultured *Glycine max* L. 'Felix' fresh weight (mg/plant). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

Effect of ZnO_2 NPs on soybean in vitro regeneration

Soybean plantlets responded differently to different concentrations of ZnO_2 NPs incorporated in the culture media. Similar to TiO_2 effects, at low concentrations the nanoparticles generated a stimulatory effect upon plant growth reaching 18.26 ± 0.57 cm as compared to control (16.56 ± 0.46 cm). Concentrations of 100 mg/l and 1000 mg/l ZnO_2 NPs inhibited plant growth and decreasing plant height by 14.32% (14.19 ± 0.29 cm) and 26.94% (12.10 ± 0.36 cm) respectively (Figure 5). Similar results have been obtained in S*tevia rebaudiana* when the presence of ZnO_2 NPs in concentrations of 0.1, 1 and 10 mg/l stimulated shoot growth, but the length of the shoots decreased as the NP concentrations increased (100 and 1000 mg/l) as compared to control (Javed et al., 2017).

At the same 10 mg/l ZnO_2 NP concentration, both average length of primary roots and secondary roots were considerably increased as compared to control. Concentrations of 100 and 1000 mg/l ZnO_2 NP reduced root development by 56.06% (3.70 ± 0.20 cm) and 75.9% (2.03 ± 0.33 cm) as compared to control (8.42 ± 0.24 cm). The same reducing pattern was observed also in the development of secondary roots (Figure 5). The average number of secondary roots was significantly lower. The increase of NPs concentrations decreased proportionally the length of secondary roots (Figure 6); thus the length of secondary roots ranged from 17.47 ± 0.54 (control) and 5.83 ± 0.36 (1000 mg/l ZnO_2 NPs). The fresh weight of the plantlets decreased in all the treatments as compared to control. It was observed that at the highest concentration of ZnO_2 NPs (1000 mg/) the average fresh weight of the plants decreased to 1061.31 ± 43.42 mg, representing a decrease of 51.77% (Figure 7). Other reports (Javed et al., 2017) show that same concentrations of ZnO_2 NPs (1000 mg/l) added to the culture media MS for *Stevia rebaudiana* led to a decrease of 56.25% from 0.16 ± 0.07 g in control to 0.07 ± 0.04 g at 1000 mg/l NPs. Concentrations of 0.1,

1, 10 and 100 ml/l ZnO_2 NPs stimulated biomass growth in Stevia.

Our results indicate that the presence of both TiO_2 and ZnO_2 NPs at the concentration of 1000 mg/l in the culture media inhibited secondary root formation and growth. Thus, it was observed that the *in vitro* plantlets emerged secondary roots only in the transition area above the level of the culture media (Figure 1c and d).

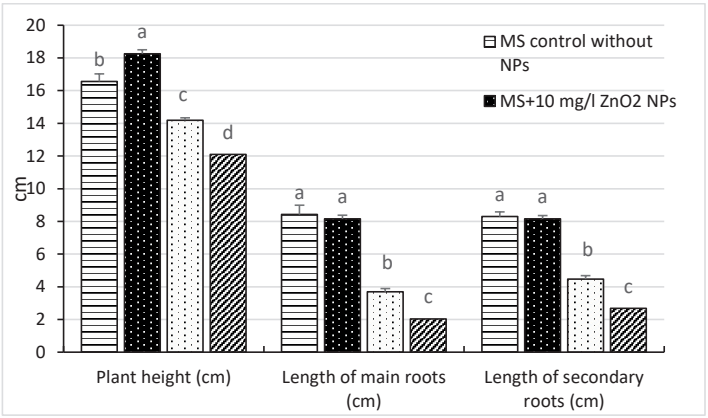

Figure 5. Effect of ZnO_2 NPs on *in vitro* cultured *Glycine max* L. 'Felix' on plant height and primary and secondary root length. The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

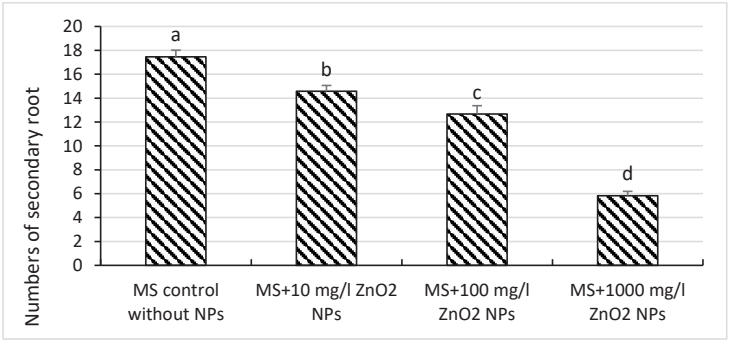

Figure 6. Effect of ZnO_2 NPs on *in vitro* cultured *Glycine max* L. 'Felix' secondary root number. The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

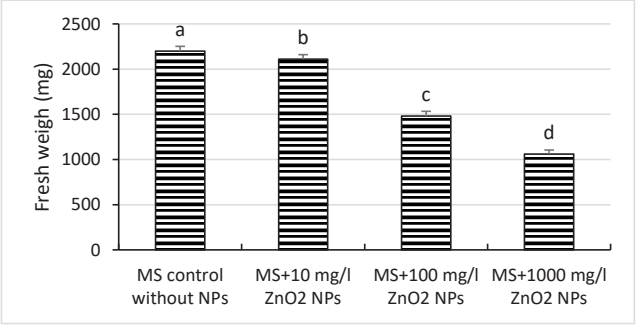

Figure 7. Effect of ZnO_2 NPs on *in vitro* cultured *Glycine max* L. 'Felix' fresh weight (mg/plant). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

CONCLUSIONS

To sum up, in this experiment we revealed the responses of *Glycine max* L. 'Felix' suggesting that TiO_2 NPs when incorporated to the culture media in concentrations of 10 mg/l or 100 mg/l did not have any negative effect on plant fresh weight or number of secondary roots. Moreover, the concentration of 10 mg/l TiO_2 NPs stimulated plant growth. Similar effects were observed also when ZnO_2 NPs were added in the same concentration simulating plant growth, but in comparison to TiO_2, ZnO_2 showed a greater inhibitory effect on the investigated plant growth parameters at concentrations of 100 and 1000 mg/l (fresh weight, number of secondary roots, length of primary and secondary roots).

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