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Effect of nanoparticles on sugar beet cultured in vitro and its betalain and betaine production

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Abstract: Sugar beet (*Beta vulgaris* L.) is considered one of the most important crops in terms of economics and food. With the widespread applications of nanoparticles (NPs) in plant science that have received great interest due to their amazing results and promising capabilities, the aim of this research was to investigate the effect of TiO_2 and ZnO NPs on sugar beet plants cultured in vitro in several respects: seed germination, morphology, chlorophyll a and b contents, carotenoids, and the most important sugar beet metabolites, betaine and betalains. To investigate the effects, four different concentrations of NPs (0, 5, 15, and 25 mg/mL) were applied to two genotypes of sugar beet. Following the applications, germination numbers and root and hypocotyl lengths were measured. Further, chlorophyll, carotenoids, betaine, and betalains contents were estimated and compared. Plants exposed to NPs did not show a significant difference in seed germination, chlorophyll contents, or carotenoids as compared to the controls. On the other hand, betaine showed improvement at low concentrations of NPs and gradually decreased with increasing concentrations in both genotypes. Concerning betaxanthin, the monogerm genotype (SG833) showed a statistically significant increase in plants exposed to TiO_2 NPs with concentrations of 15 and 25 mg/mL, while in the multigerm genotype (SG2020), a significant improvement was shown in plants exposed to ZnO NPs at concentrations of 15 and 25 mg/mL. Regarding betacyanin, it showed a statistically significant enhancement only in plants exposed to 25 mg/mL ZnO NPs in the monogerm genotype. The effects of ZnO and TiO_2 NPs at varying concentrations on sugar beet were studied. The research focused on the production of betaine and betalains, which are important secondary metabolites. The results indicated the possibility of increasing the production of these compounds while avoiding any negative or phytotoxic effects.

Key words: Sugar beet, in vitro, nanoparticles, betaine, betalain

1. Introduction

Sugar beet (*Beta vulgaris* L.) is a significant root crop cultivated all over the world and used for sugar extraction, producing millions of tons of sugar for human use and beet pulp for animal feed each year (Shokohian and Omidi, 2021). In 2019, the world produced approximately 278 million tons of sugar beet.[1] Previously, in 2018, about 42 million tons of sugar was produced from sugar beets globally, which represents approximately 30% of the global sugar supply (Lv et al., 2019). Türkiye ranks fifth in the world in sugar beet production, after France, the Russian Federation, the United States, and Germany, according to the Food and Agriculture Organization of the United Nations. In Türkiye, 6,007,777 tons, or 29% of the total sugar beet production (17,436,100 tons), is produced in Konya Province (Avan et al., 2020). Moreover, sugar beet production in 2022 in Türkiye increased by 6.9% to 19 million tons.[2] This noticeable difference in sugar beet production shows that it has been exploited in the best possible ways for many years due to its importance and benefits. The allure of sugar beets transcends their actual production potential since they contain a remarkable array of secondary metabolites. It is one of the few edible plants that contain betalains, a class of bioactive secondary metabolites with a variety of biological activities and possible health advantages (Clifford et al., 2015). Betalains are nitrogen-containing vacuolar pigments that are water soluble (Rodriguez-Amaya, 2019) and are natural pig-

1 Shahbandeh M (2021). Sugar Beet Production Worldwide from 1965 to 2019 [online]. Statista website. https://www.statista.com/statistics/249609/sugar-beet-production-worldwide/ [accessed 17.01.2023]

2 Türkiye İstatistik Kurumu (2022) [online] Bitkisel üretim istatistikleri, 2022. Website: https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2022-45504 (in Turkish) (accessed 17.01.2023).

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ments primarily consisting of yellow-orange betaxanthins or red-violet betacyanins, which are betalamic acid derivatives, in addition to being natural colorants. As it is known that free radicals cause many disorders, betalain, with its antioxidant and antiradical effects, plays an important and healthy role in maintaining life and resisting stress. Furthermore, betalains have anticancer, antiviral, hepatoprotective, antibacterial, and antiinflammatory properties, as well as gut- and immune-regulating properties (Rahimi et al., 2019).

Moreover, sugar beet is distinguished by the accumulation of glycine betaine. This feature is particularly significant since sugar beet has salt tolerance, with glycine betaine playing a vital role in this capacity. Knowing that young leaves accumulate more betaine than mature leaves, betaine in sugar beet molasses has become economically valuable (Preedy, 2015). The main function of betaine is to protect cells from osmotic disruption (Craig, 2004). Furthermore, it contributes to the health of the liver, heart, and kidneys. In turn, as a methyl donor, it has the potential to lower elevated blood homocysteine concentrations, and it may also play a role in epigenetics and athletic performance (Craig, 2004). Addressing the salinity tolerance of key crop plants is a pressing worldwide issue. The impact of soil salinity on plant growth, stemming from osmotic stress and ion-specific toxicity, is widely recognized. This underscores the significance of osmotic agents like betaine, which occur naturally in specific plant species like sugar beet (Preedy, 2015). This is one of the reasons for the expansion of the beet crop and its spread in many countries around the world, and it is constantly being developed and studied.

Nanotechnology is one of the new areas integrated into agricultural applications, as interdisciplinary studies are increasing rapidly. Because of their unique optical features, high surface-to-volume ratio, and size-dependent characteristics, nanomaterials show considerable promise for use in nutrition and plant protection (Nair et al., 2010). Nanoparticles (NPs) have been used widely in plant tissue culture studies. NPs can be used to promote or enhance the morphogenetic potential of explants obtained from different plant species (Koçak et al., 2023).

The impact of NPs on plants can be advantageous or detrimental, ranging from overt physiological changes to subtle biochemical alterations. Phytonanotechnology, or the combination of plant systems and nanotechnology, presents potentially revolutionary opportunities. The complex interaction between plants and nanomaterials has the ability to alter how plants grow, develop, and react to stress, emphasizing the need to cautiously explore their potential benefits while addressing any risks in order to achieve successful outcomes in agriculture and crop production (Verma et al., 2018, 2019).

The most common nanomaterials produced globally are zinc oxide (ZnO) and titanium dioxide (TiO_2). ZnO NPs have a wide range of applications due to their strong photochemical activity and high catalytic activity, as well as their distinct antifungal and antibacterial properties (Sirelkhatim et al., 2015). Moreover, data show that the accumulation of ZnO NPs decreased pathogen activity in *Beta vulgaris*, thereby suppressing illness (Khan and Siddiqui, 2021a). The effect of ZnO NPs on crop plants has been extensively assessed. The toxic effects of ZnO NPs observed in some plants treated with ZnO NPs are caused mainly by the suppression of genes involved in chlorophyll synthesis and the structure of the photosynthetic system (Wang et al., 2016). Furthermore, the efficacy of plants in removing heavy metals from contaminated water can be enhanced by nanotreatment with ZnO NPs at optimum concentration, and this is remarkable, indicating that the use of ZnO NPs can reduce the intake of heavy metals while having a favorable impact on the accumulation or translocation of other nutrients (Hussain et al., 2021).

One of the most widely produced nanomaterials worldwide is TiO_2 NPs, which were found to stimulate RuBisCO activity, increase photosynthetic rate, and prevent chloroplasts from aging during lengthy illumination regimes (Yang et al., 2006). Additionally, the antioxidant defense mechanism of *Beta vulgaris* was activated by TiO_2 NPs, which in turn decreased disease indices (Khan and Siddiqui, 2021b). Further, there is considerable evidence that TiO_2 NPs help crops grow and become more resilient to abiotic stressors (Choi et al., 2015). All these characteristics of TiO_2 NPs are the reason for the increasing interest in them, the escalation of their manufacture and production, and the tireless attempts to employ them more and more in agricultural applications.

The function of NPs as secondary metabolite elicitors is highly dependent on their chemical compositions and physical properties, as well as the concentration of application; therefore, extensive dose-response studies must be conducted to determine the optimum concentration for each NP–plant system to maximize secondary metabolite yield while minimizing toxic effects on the plant, the consumers, and the environment (Lala, 2021).

The present study centers on investigating the impact of zinc oxide and titanium dioxide NPs on two genotypes of sugar beet plants. This plant holds significant agricultural value globally, and especially in Türkiye. The study delves into many aspects, such as seed germination and physical changes caused by NPs. The primary objective of the study was to determine the potential impacts of TiO_2 and ZnO NPs on various aspects of sugar beet plants. These include seed germination, physical characteristics, and levels of chlorophyll and carotenoids, as well as the synthesis of betaine and betalains. The overarching aim was to evaluate

whether these NPs can significantly influence the growth, development, and metabolite generation of sugar beets. The research also explored possible variations in how different genotypes respond to NP treatments. This may provide opportunities for NPs to contribute to enhancing secondary metabolites and thus improving the quality of agricultural crops and increasing their stress tolerance.

2. Materials and method

2.1. Plant material

Two genotypes of sugar beet seeds were used**:** SG833, a monogerm line, and the SG2020 multigerm line. Both of them were developed at the Sugar Institute.

2.2. Sterilization and germination of sugar beet seeds

Seeds of the two sugar beet genotypes were surface sterilized with 70% EtOH for 5 min, followed by treatment with 70% commercial bleach and Tween 20 (0.5 mL/100 mL) for 60 min. After the seeds were rinsed, they were soaked with sterile dH_2O overnight at 25 °C. The water was decanted and the seeds were rinsed with a 5% PPM solution (Plant Cell Technology Inc., Washington, DC, USA).

After sterilization, the sugar beet seeds were kept in NP solutions for 24 h, with the solution containing different concentrations (5, 15, and 25 mg/mL) of ZnO (Sigma-Aldrich, Cat No: 721077) and TiO_2 (Sigma-Aldrich, Cat No: 700347), while the control seeds were kept in sterile dH_2O for 24 h. The sizes of the NPs are given by the manufacturer as <100 nm (TEM) and ≤40 nm average particle size (APS) for ZnO and for TiO_2 the size is given as <150 nm (volume distribution, DLS). Afterward, these seeds were germinated in MS (Murashige and Skoog, 1962) nutrient medium, containing the same concentrations of the relevant NPs, in Petri dishes. The seeds were kept at 23 ± 1 °C in the dark for 5 days before being switched to a 16-h light cycle with an 8-h dark photoperiod at a relative humidity of 55%–60% until they were 7 days old after germination. These 7-day-old seedlings were then transferred to sterile glass jars containing MS nutrient medium with NPs in the same concentrations. The sugar beet seedlings were harvested 4 weeks after germination and stored at –80 °C.

2.3. Estimation of the growth and development of plants with different parameters

The germination rate (%) was recorded from the first day of germination and updates were added on a daily basis.

The morphological effects (root and hypocotyl lengths) of different NP treatments on 4-week-old sugar beet plants were compared with those on the control group.

2.4. Determination of chlorophyll and carotenoid contents

Chlorophyll (C_a, C_b), and carotenoids (C_{xc}) were analyzed and calculated as described by Lichtenthaler and Welburn (1983). The absorbance was measured by a UV spectrophotometer at 470, 649, and 665 nm.

2.5. Determination of betaine contents

The estimation of betaine content was carried out using the following protocol (Valadez et al., 2016) with modifications: 250 mg of each sample was ground and completed to 5 mL with dH_2O. The samples were incubated for 24 h at 25 °C. After that, they were placed in a centrifuge (10,000 rpm) for 15 min at room temperature, and then 2 N sulfuric acid (Merck KgaA, Cat No. 1.00731.2500) at a 1:1 ratio was added to the supernatant. After 1 h of cooling in an ice bath, 100 mL of potassium iodide–iodine solution was added to each tube. The samples were kept at 0 °C for 16 h and betaine crystals formed. The tubes were centrifuged (10,000 rpm) at 0 °C for 15 min. The betaine crystals were collected and dissolved in 4.5 mL of 1,2-dichloroethane (Fluka Analytical, Cat No: 03530). The samples were incubated for 2 h at room temperature and spectrophotometrically measured at a wavelength of 225 nm. The concentrations of the betaine contents were compared as percent changes against the control.

2.6. Determination of betalain contents

The estimation of betalain content was carried out with the following procedure (Lazăr et al., 2021): 500 mg of each sample was ground and 1.5 mL of citric acid solution (1.5 g of citric acid with 50% ethanol) was added. The samples were then gently rotated and homogenized for 15 min in an ice bath, and then the samples were incubated at 52 °C for 50 min. Immediately thereafter, ultrasonification was applied to homogenize the samples for 5 min; then the samples were centrifuged (10,000 rpm) at room temperature for 15 min. Absorbance readings were taken from the supernatants at 538, 476, and 600 nm. Although there are other chemicals similar to betanin and vulgaxanthin I, they represent 95% of the betalain content. The following equations were used to determine the concentrations (Skalicky et al., 2020):

$$A_{Betanin} = 1.095 \times (A_{538} - A_{600})$$

$$A_{Impurities} = A_{538} - A_{Betanin}$$

$$A_{Vulgaxanthin\ I} = (A_{476} - A_{Impurities} - A_{Betanin}) / 3.1$$

$$C_{Betanin} = (25 \times A_{Betanin}) / 1120$$

$$C_{Vulgaxanthin\ I} = (25 \times A_{Vulgaxanthin\ I}) / 750$$

2.7. Statistical analysis

All results were presented as mean ± standard deviations. The data were analyzed by one-way ANOVA, and the mean of the results was compared with the mean of the controls with GraphPad Prism. Dunnett's test, which compares means of the treatments against the control group, was used to evaluate the findings. P values were given as 0.12 (ns), 0.033 (*), 0.002 (**), and 0.001 (***).

3. Results

3.1. Seed germination analysis

The two plant genotypes, SG833 and SG2020, had different natural germination rates, where the multigerm (SG2020) genotype had a higher germination rate than the monogerm genotype (SG833). Such differences can arise due to inherent genetic differences. However, all treatments with NPs (TiO_2 and ZnO) did not noticeably alter the germination process in either genotype. This implies that the NPs had a similar impact on seed germination as the normal conditions, acting similarly to the control group for both types of seeds (Figure 1).

3.2. Comparing the phenotypic changes in plants

The effects of TiO_2 and ZnO NPs did not differ in the treatments applied to the morphology of 4-week-old sugar beet plants in terms of root length or hypocotyl length compared to the control plant group in either genotype (Figure 2).

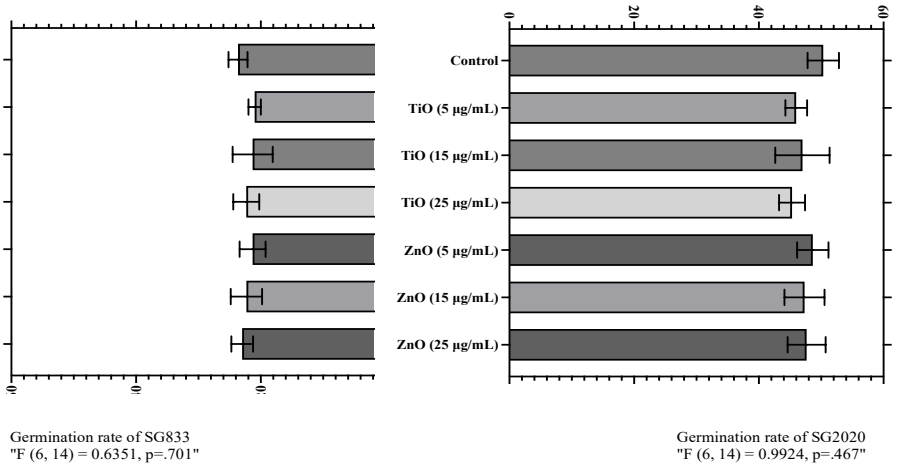

Figure 1. The effect of TiO_2 and ZnO NPs on the germination rates of the genotypes SG833 and SG2020 at 5, 15, or 25 mg/mL concentrations (0.12 (not given in the figure), 0.033 (*), 0.002 (**), 0.001 (***)).

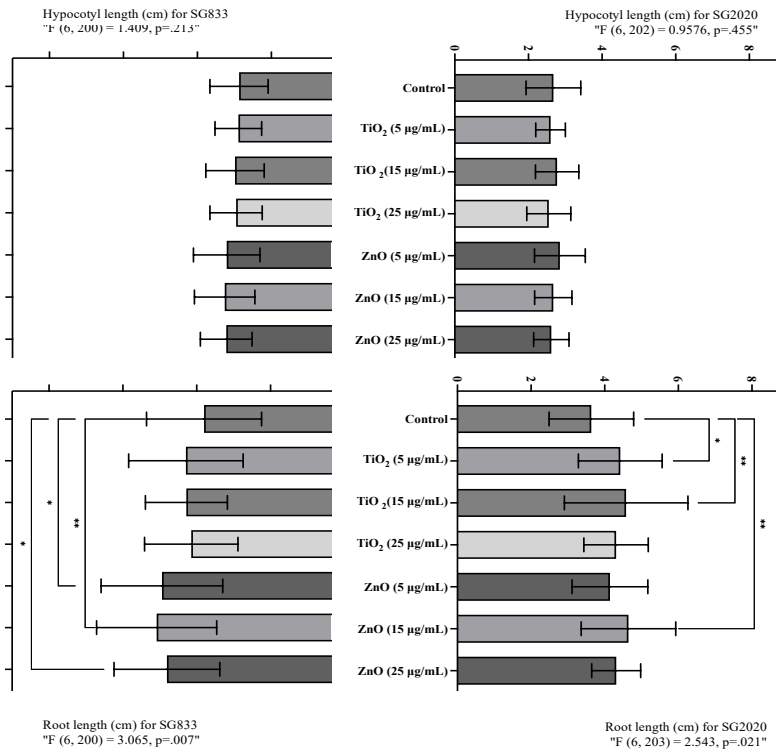

Figure 2. The effect of TiO_2 and ZnO NPs at 5, 15, or 25 mg/mL concentrations on the plant phenotype (roots length and hypocotyls length) in both genotypes SG2020 and SG833 (0.12 (not given in the figure), 0.033 (*), 0.002 (**), 0.001 (***)).

3.3. Determination of chlorophyll a, chlorophyll b, and carotenoids

In comparison to the control plant groups, both types of TiO_2 and ZnO NPs and all concentrations applied caused an improvement in chlorophyll a and b production, but according to the statistical analysis, these results were not significant. However, there were no significant changes in the carotenoid levels (Figures 3 and 4).

3.4. Estimation of betalains (betanin and vulgaxanthinI)

The effect of NPs on betanin (a component of betalain derived from betacyanin) in the genotype SG833 plants showed a clear and statistically significant increase only in plants exposed to 25 mg/mL ZnO NPs. As for vulgaxanthin I (which is derived from betaxanthin, the other component of betalains), the effect of TiO_2 and ZnO NPs on plants showed a significant improvement only in plants that were exposed to NPs of TiO_2 at a concentration of 15-25 mg/mL compared to the control plants. Genotype SG2020 plants showed no difference in betanin production compared to the control plants. As for vulgaxanthin I, it showed a positive response and improvement in plants exposed to ZnO NPs at concentrations of 15 and 25 mg/mL compared to the control plants (Figure 5).

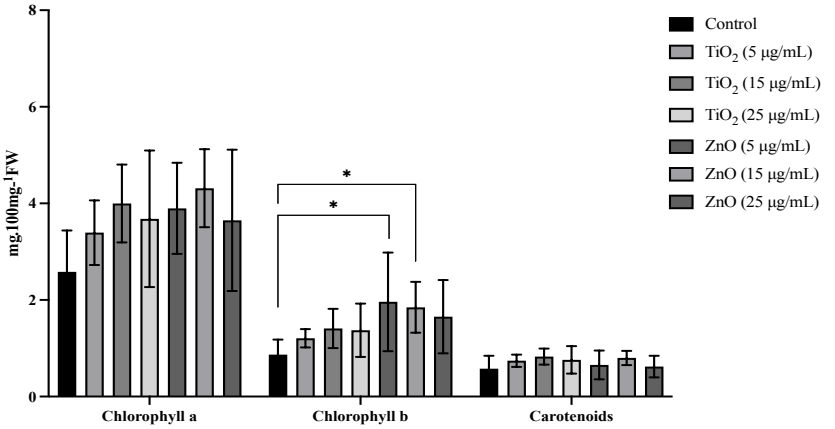

Figure 3. Effect of TiO_2 and ZnO NPs at 0, 5, 15, or 25 mg/mL concentrations on chl a, chl b and carotenoids production in the genotype SG2020. For Chlorophyll a: F (6, 35) = 1.707, p = 0.148; Chlorophyll b: F (6, 35) = 2.414, p = 0.047; Carotenoids: F (6, 35) = 1.056, p = 0.407 (0.12 (not given in the figure), 0.033 (*), 0.002 (**), 0.001 (***)).

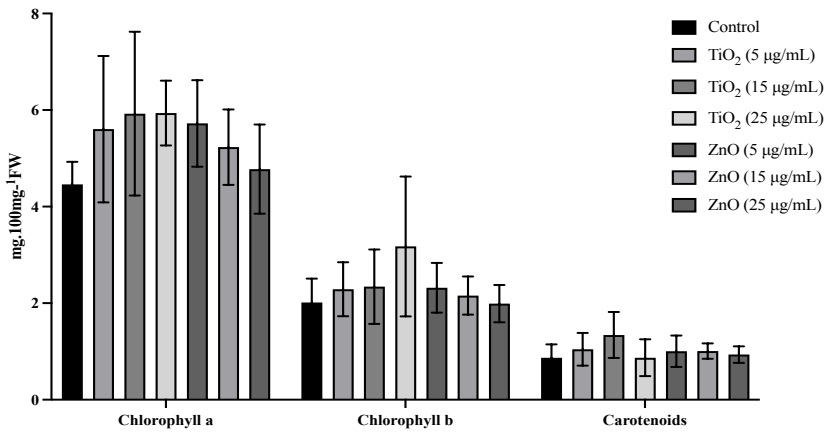

Figure 4. Effect of TiO_2 and ZnO NPs at 0, 5, 15, or 25 mg/mL concentrations on chl a, chl b and carotenoids production in the genotype SG833. For Chlorophyll a: F (6, 35) = 1.735, p = 0.142; Chlorophyll b: F (6, 35) = 1.761, p = 0.136; Carotenoids: F (6, 35) = 1.505, p = 0.205 (0.12 (not given in the figure), 0.033 (*), 0.002 (**), 0.001 (***)).

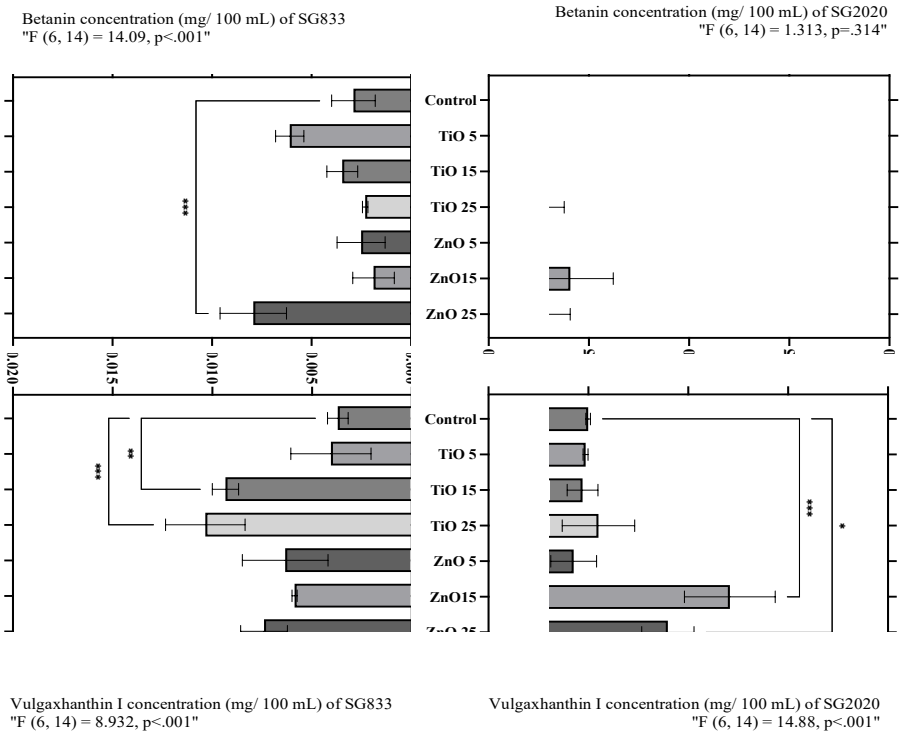

Figure 5. Effect of TiO_2 and ZnO NPs at 0, 5, 15, or 25 mg/mL concentrations on betanin and vulgaxanthin I production in both genotypes SG2020 and SG833 (0.12 (not given in the figure), 0.033 (*), 0.002 (**), 0.001 (***)).

3.5. Estimation of betaine

For the relative comparison of betaine contents, the control group was used as the baseline, with a concentration of zero, and the percentages display the rise or decrease in comparison to this baseline. In the SG2020 genotype, an increased concentration of betaine was measured in plants exposed to TiO_2 NPs and ZnO NPs compared to the control group (Figure 6a). The highest concentration of betaine was found with TiO_2 5 mg/mL, reaching 282.64%, and gradually decreasing at TiO_2 15 mg/mL to 240.27% and 85.56% at a TiO_2 concentration of 25 mg/mL, compared to the control group. As for ZnO NPs, they reached the highest concentration at 5 mg/mL with a value of 232.03% and also decreased gradually at ZnO 15 mg/mL to 163.32% and then 99.09% at ZnO 25 mg/mL compared to the control group. Likewise, the results for the SG833 genotype were consistent with previous results for SG2020 (Figure 6b). The highest concentration of betaine at the concentration of 5 mg/mL of TiO_2 NPs reached 33.27% and gradually decreased to 28.91% at the concentration of 15 mg/mL; then it was the lowest value of 4.27% at concentration of 25 mg/mL of TiO_2 NPs, with respect to the control plants. Regarding ZnO NPs, the content of betaine according to the control plants was 29.88%, 18.8%, and 4.02% in plants at concentrations of 5, 15, and 25 mg/mL, respectively.

4. Discussion

In terms of germination rate and morphology (the roots and hypocotyls), there was no significant difference between the control plants and the plants to which the NPs were applied. The current study's results indicate that TiO_2 and ZnO NPs have no negative effects on germination, the development of shoots, or root growth. This could be due to the plant's insensitivity to these chemicals (Ramesh et al., 2014).

Seed germination represents the first interface between the plant system and NPs; consequently, seed germination rate is a crucial parameter for assessing the impact of various nanomaterials on the subsequent developmental stages of plants (Sheikh Mohamed and Sakthi Kumar, 2016). Many studies have focused on this aspect, both negatively and positively.

Interestingly, the application of low concentrations of ZnO NPs has been linked to enhanced seed germination in several plant species, such as peanuts (*Arachis hypogea*). The precise cause of these effects is unknown; however, it is possible that they are related to the potential fertilizer-like role that zinc in seeds plays after treatment with ZnO NPs; they are also caused by the NPs' low bioavailability due to their size and low water solubility, in addition to the fact that the inherent small size and large surface area associ-

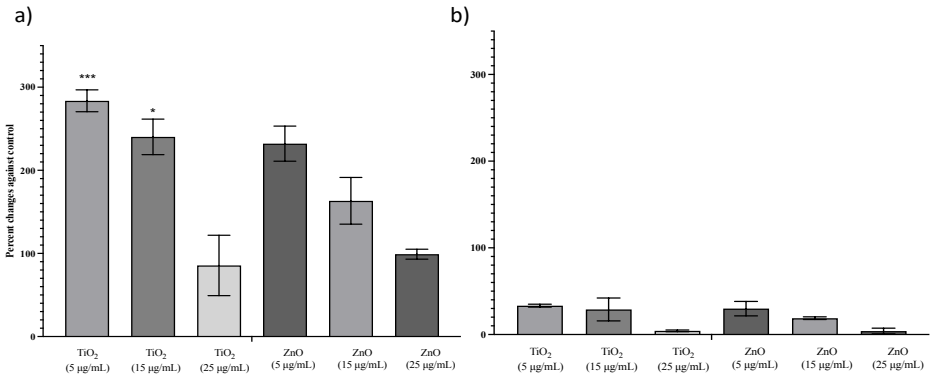

Figure 6. a) Percent changes in betaine concentrations in different NP applications for genotype SG2020 against the control (F (6, 47) = 14.46, p < 0.001). **b**) Percent changes in betaine concentrations in different NP applications for the genotype SG833 against the control. (F (6, 40) = 1.391, p = 0.242) (0.12 (not given in the figure), 0.033 (*), 0.002 (**), 0.001 (***)).

ated with ZnO may lead to increased adsorption (Prasad et al., 2012).

Germination rates may differ significantly depending on exposure time, medium concentrations, and the plant species being tested. This implies that the variation in NP size influences phytotoxicity, but varietal variations may also be responsible for the results (Rosa et al., 2013). Furthermore, the various impacts of NPs on germination can be attributable to differences in cultivar composition as well as size and solubility. The interactions between NPs and plants are complex, necessitating extensive biochemical and analytical research. When exposed to ZnO NPs, zucchini seeds showed no adverse impacts on germination or root growth (Stampoulis et al., 2009). On the other hand, another study investigated the impact of TiO_2 NPs on seed germination and plant growth using amaranth and cruciferous vegetables. They showed that the effect of TiO_2 NPs on plants is dosage-dependent. At lower concentrations, TiO_2 NPs activity positively influenced seed germination and growth by modulating gibberellin levels, promoting plant development within tolerable limits. However, at higher concentrations, TiO_2 NPs resulted in tissue damage. This balance is vital for achieving sustainable development and offers a promising avenue for agricultural applications (Li et al., 2022).

Eventually, the nonadverse effects of NPs on plant growth, as well as the root and hypocotyl lengths in the present study, indicate that the application concentrations, NP sizes, experimental procedures, conditions, and duration of exposure chosen are appropriate and have no adverse effects on plant growth.

For both genotypes, chlorophylls and carotenoids were unaffected by the application of TiO_2 and ZnO NPs at any concentration. One of the most important studies showing the difference in response between plants under the influence of the same type of NPs was a comparison between nine different plants and the effect of zinc on chlorophyll a and b. The results varied between the studied plants, and the authors explained that although many studies have shown that ZnO NPs can induce oxidative stress and modulate the activity of antioxidant enzymes or chloroplast function, their study revealed that ZnO NPs induced changes in several oxidative stress-related parameters across the majority of the plant species investigated. However, the extent of these changes varied significantly depending on the plant species (García-Gómez et al., 2018).

In another study, the effect of ZnO NPs on *Triticum aestivum* was observed with a decrease in oxidative stress and a high level of chlorophyll and carotenoids (Hussain et al., 2018). Similarly, the role of TiO_2 NPs in reducing oxidative stress and increasing chlorophyll and carotenoid content was demonstrated in the rice plant (Rizwan et al., 2019). As a result, the pathways associated with the effects of NPs on plants are distinct and overlap, which is consistent with the findings of the present study in terms of the variation in outcomes across all parameters.

Despite the relatively restricted exploration into the effects of NPs on betaine and betalain, investigations have delved into other secondary metabolites. One study found that ZnO NPs had a positive impact on in vitro steviol glycoside production in *Stevia rebaudiana* and by using 1 mg/L of ZnO NPs, the amount of steviol glycosides obtained was nearly doubled compared to the control (Javed et al., 2017).

Numerous studies have shown the efficiency of ZnO NPs by boosting secondary metabolites in *Momordica charantia* L., including phenols, flavonoids, carotenoids, proline, and carbohydrates (Sharifi-Rad et al., 2020).

The precise mechanism by which NPs accelerate secondary metabolism is unknown. One possible mechanism is that NPs generate reactive oxygen species (ROS), then triggering secondary metabolism to deal with the stress caused by ROS. Another method of increasing their production is to increase the expression of various genes involved in secondary metabolite synthesis. Significantly, NPs have been associated with plant toxicity resulting from the production of ROS. Considering the interplay between ROS and secondary signaling, which impacts the regulation of secondary metabolites at the transcriptional level, the ROS generated by NPs might play a role in stimulating secondary metabolism in plants (Marslin et al., 2017). Although NPs have long been recognized for inducing abiotic stress and toxicity in plants, they are now emerging as tools for stimulating beneficial secondary metabolites both within plant systems and as agents for extracting metabolites from plant cells (Rivero-Montejo et al., 2021). Notably, the functions of NPs as secondary metabolite elicitors are highly dependent on their chemical composition, size, and shape, as well as the concentration of application. It is noteworthy that the synthesis of secondary metabolites can be stimulated and enhanced by NPs, but in a dose-dependent manner, and so extensive dose-response studies must be conducted to determine the optimum concentration for each NP–plant system in order to maximize secondary metabolite yield while minimizing negative effects on the plant, the consumers, and the environment (Lala, 2021). In summation, the investigation into the effects of TiO_2 and ZnO NPs on two genotypes of sugar beet plants has unraveled a fascinating interplay between nanomaterials and plant physiology. The results of this research offer promising indications of NPs' capacity to boost growth, trigger secondary metabolite production, and potentially serve as tools for extracting metabolites. The nonadverse effects on germination, root and shoot development, and the chlorophyll and carotenoid content underline the appropriateness of the experimental parameters chosen.

With the important role of betaine and betalain as secondary metabolites, this research highlights the potential for their increase. NPs, formerly regarded as possible threats, are now displaying their ability to favorably transform the agricultural landscape. To realize this promise, comprehensive dose-response studies, meticulous understanding of biochemical pathways, and a dedication to environmental stewardship are required. It also increases the possibility of building a greener and more resilient world, one in which our food demands and environmental goals are harmoniously met.

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