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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Biology

IN VITRO EFFECT OF NANOPARTICLES ON SUGAR BEET (BETA VULGARIS) AND ITS SECONDARY METABOLITES (BETALAIN AND BETAINE) PRODUCTION

MASTER OF SCIENCE

NOUR ALAWAD

ACADEMIC SUPERVISOR
Prof. Dr. EKREM GÜREL

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APPROVAL OF THE THESIS

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Nour Alawad

ABSTRACT

IN VITRO EFFECT OF NANOPARTICLES ON SUGAR BEET (BETA VULGARIS) AND ITS SECONDARY METABOLITES (BETALAIN AND BETAINE) PRODUCTION
MSC THESIS
NOUR ALAWAD
BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF. DR. EKREM GÜREL)

BOLU, APRIL 2023
(Xiii + 57)

Sugar beet (*Beta vulgaris* L.) is considered one of the most demanded and high economic value food crops. Many studies have been done on the development of this plant. With the widespread applications of nanoparticles in the field of plants that have received great interest, due to their amazing results and promising capabilities that are considered so far only the "tip of the iceberg", this research was conducted to investigate the effect of two nanoparticles on the sugar beet plants in several respects; seed germination, morphology, chlorophyll A and B content, and carotenoids, as well as two of the most important sugar beet metabolites, betaine and betalain. TiO_2 NPs and ZnO NPs were applied in four different concentrations separately (0, 5, 15, and 25 mg/ml) on two genotypes of sugar beet plants, monogerm (SG833) and multigerm (SG2020), by adding the NPs to the MS medium. Using a UV-spectrophotometer, chlorophyll, carotenoids, betaine, and betalain contents were estimated. Plants exposed to NPs did not have a significant difference in terms of seed germination, chlorophyll contents, or carotenoids compared to the control. In comparison to the control group, it showed a statistically significant difference and improvement for betaine at low concentrations of NPs and gradually decreased with increasing concentrations in both genotypes. In contrast to the multigerm genotype (SG2020), which showed a statistically significant increase in the content of betaxanthin at a concentration of 15 mg/ml ($p<0.01$) and 25 mg/ml ($p<0.05$) of ZnO NPs compared to the control. Regarding betacyanin, only the plants exposed to ZnO NPs at the highest concentration, which is 25 mg/mL, showed a statistically significant increase ($p< 0.05$), compared to the control plants. It is indicated that the response of plants during exposure to NPs depends on several factors, including the type of nanoparticles, their concentration, and the exposure time; moreover, it varies between plant species and even between genotypes within the same plant species.

KEYWORDS: Sugar beet, nanoparticles, ZnO, TiO_2, betaine, betalain, UV-spectrophotometer.

ÖZET

NANOPARTİKÜLLERİN ŞEKER PANCARI (BETA VULGARIS) VE İKİNCİL METABOLİTLERİ (BETALAİN VE BETAİN) ÜRETİMİ ÜZERİNDEKİ İN VİTRO ETKİSİ
YÜKSEK LİSANS TEZİ
NOUR ALAWAD
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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
BİLİM DALI (BİYOLOJİ ANABİLİM DALI)
(TEZ DANIŞMANI: PROF. DR. EKREM GÜREL)

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Şeker pancarı (*Beta vulgaris* L.), en çok talep edilen ve yüksek ekonomik değere sahip gıda ürünlerinden biri olarak kabul edilir. Bu bitkinin geliştirilmesi üzerine birçok çalışma yapılmıştır. Şaşırtıcı sonuçları ve şimdiye kadar sadece "buzdağının görünen kısmı" olarak kabul edilen umut verici özellikleri nedeniyle büyük ilgi gören nanopartiküllerin bitkilerdeki yaygın uygulamaları dikkate alınarak, bu araştırma, şeker pancarı bitkilerinde tohum çimlenmesi, morfolojisi, klorofil a ve b içeriği ve karotenoidler ile en önemli şeker pancarı metabolitlerinden ikisi olan betain ve betalain üretimi üzerine iki farklı nanopartikülün etkisini araştırmak için yapılmıştır. TiO_2 ve ZnO NP'lerin dört farklı konsantrasyonu (0, 5, 15 ve 25 mg/ml) MS besin ortamına eklenerek, bir monogerm (SG833) ve bir multigerm (SG2020) şeker pancarı genotipine ait bitkilere uygulanmıştır. UV-spektrofotometre kullanılarak klorofil a ve b, karotenoidler, betain ve betalain içerikleri belirlenmiştir. NP'lere maruz kalan bitkiler, kontrol ile karşılaştırıldığında tohum çimlenmesi, klorofil içeriği veya karotenoidler açısından önemli bir fark gözlenmemiştir. Kontrol grubuyla karşılaştırıldığında, her iki genotipte ölçülen betain düzeyi düşük NP konsantrasyonlarında istatistiksel olarak anlamlı bir fark gözlenmiş, artan konsantrasyonlarda ise kademeli olarak azalmıştır. Multigerm genotipin (SG2020) bitkilerine ZnO NP 15 mg/ml ($p<0.01$) ve 25 mg/ml ($p<0.05$) konsantrasyonunda uygulandığında, betaksantin içeriğinde istatistiksel olarak anlamlı bir artış gözlenmiştir. Betasiyanin ile ilgili olarak, sadece en yüksek konsantrasyon olan 25 mg/ml'lik ZnO NP'lere maruz kalan bitkiler, kontrol bitkileriyle karşılaştırıldığında istatistiksel olarak anlamlı bir artış ($p<0.05$) göstermiştir. NP'lere maruz kalma sırasında bitkilerin tepkisinin, nanopartiküllerin türü, konsantrasyonları ve maruz kalma süresi dahil olmak üzere çeşitli faktörlere bağlı olduğu, ayrıca bitki türleri arasında ve hatta aynı bitki türü içindeki genotipler arasında farklılık gösterebilceği belirtilmektedir.

ANAHTAR KELİMELER: Şeker pancarı, nanopartiküller, ZnO, TiO_2, betain, betalain, UV-spektrofotometre.

To all the displaced, refugees, and people in shelter tents who left their homes, dreams, and so much of their lives behind and started over somewhere else with new dreams, identities, features, and privileges, you are the greatest; you always prove that dreams have no boundaries and are a right for all.

You can definitely be more than just a survivor.

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LIST OF ABBREVIATIONS AND SYMBOLS

NPs **:** Nanoparticles

TiO2 **:** Titanium dioxide

ZnO **:** Zinc oxide

ROS **:** Reactive oxygen species

FAO **:** Food and Agriculture Organization of the United Nations

TEM **:** Transmission electron microscopy

SEM **:** Scanning electron microscop

GB **:** Glycine betaine

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1. INTRODUCTION

1.1 Sugar beet (*Beta vulgaris L.)* overview:

Beta vulgaris L. ssp. is the scientific name for sugar beet. The most common sugar beet variety (1). There are four sections within the genus Beta L., which belongs to the Amaranthaceae (earlier Chenopodiaceae) family (2). It is a significant root crop grown all over the world and used for sugar extraction. Each year, millions of tonnes of sugar for human consumption and beet pulp for animal feed are produced by the sugar beet, the world's second most important source of sugar after sugar cane (3).

About 42 million metric tons of sugar from sugar beets were produced globally in 2018, making up close to 30% of the global sugar supply (FAO) (4). Six years before that, in 2012, 20% of the world's sugar needs were met by sugar beet (5). 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.

Because sugar beet is known to be a salt-tolerant crop, it is an ideal study subject for salt acclimation in plants (4). Sugar beet may be produced on marginal land since they are very tolerant of many climatic conditions and soil types. Selection criteria used in plant breeding programs include yield, disease and pest resistance, sugar content, and nutrition (6). The whole beet can also be used as a feedstock for animals or as a source of alcohol thanks to its coproducts of greens, molasses, and pulp residue (7).

High-quality sugar beet hybrid seeds take several years of experimentation and thorough examination of many plants, so exclusive nurseries and some sugar beet companies produce e beet seeds; however, there are two types of seeds: mono-seeded (monogerm) and multi-seeded (multigerm), the monogerm seeds being more popular among growers (8).

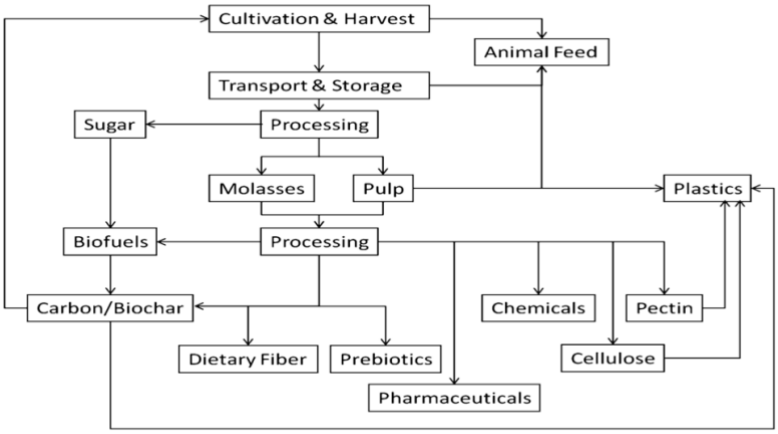

Figure 1.1. Schematic of complete utilization of sugar beet (5).

High in nutrients, sugar beet is mostly farmed for food purposes rather than for the creation of sugar (9). It is a abundant source of phytochemicals like ascorbic acid, phenolic acids, carotenoids, and flavonoids (10). Besides that, it is one of the few plants to contain betalains (11), a group of bioactive secondary metabolites with a range of biochemical functions and potential health benefits (12). Additionally, *Beta vulgaris* is distinguished by the accumulation of glycine betaine. (13).

The genus Beta is divided into four divisions in accordance with the nomenclature of Ford-Lloyd & Williams: Beta (formerly known as Vulgares), Corollinae, Nanae, and Procumbentes (14).

1.1.1 History of *Beta vulgaris*:

The sea beet (*Beta vulgaris* L. ssp. *maritima* (L.)), a plant that is indigenous to the Mediterranean region and the coasts from Morocco up to the North Sea, is the progenitor of all beet crops (15). The ability to extract sugar from beets was one of the most significant agricultural advances in Northern Europe during the nineteenth century. German chemists discovered that sugar derived from beet roots had crystals that resembled sugar cane crystals. In the latter half of the eighteenth century, many attempts were made to produce the crop in the fields. Only since the beginning of the nineteenth century, in 1802, has it been possible to extract sugar

from sugar beets (7). After that, the sugar beet industry has grown significantly and expanded dramatically.

1.1.2 *Beta vulgaris* in Turkey:

Turkey ranks fifth globally in terms of sugar beet output, behind France, the Russian Federation, the United States, and Germany, according to the Food and Agriculture Organization of the United Nations. The 6,007,777 tons, or 29% of Turkey's total sugar beet production—17,436,100 tons—are produced in Konya Province (16). Farmers and gardeners both grow landraces of leaf beet and beet roots, which come in many varieties and landraces and have been farmed and utilized locally in Turkey for many years (17).

1.1.3 Condition of growth:

Although sugar beet can survive in both extremely cold and warmer climates, it favors colder environments for growth. It thrives almost everywhere in Europe, from the southern temperate country of Turkey to the northern cold countries of Sweden, Denmark, and Finland (8).

In addition to being widely dispersed geographically, the crop grows well on a variety of soils. The crop can be found on almost every type of soil, including clays, silts, sands, and organic soils. In the spring and fall, soils with high moisture may decrease production (7).

1.2 Nanoparticles:

Nanotechnology is one of the newest technologies that has impacted practically every aspect of our lives and is applied in agriculture production. All areas of research have now seen their perspectives broadened by nanotechnology. They are extensively utilized in industrial goods, medicines, engineering disciplines, healthcare, and agriculture (18).

Over the past ten years, nanotechnology has become a promising field with numerous applications. The broad term "nanomaterials" for nanoscale systems has found several uses in bringing innovation to many biological processes. Research has uncovered countless uses for nanomaterials, including nanoparticles,

nanosheets, nanofilms, nanorings, nanorods, and nanotubes, among many other forms. Nanoparticles are the best-known subset of nanomaterials (19).

The term "nanoparticles" refers to solid or liquid particles or dispersions with a dimension between 10 and 1000 nm (20). They are considered the fundamental units in nanotechnology.

These particles have been used by numerous human industries for thousands of years, but recently have seen a resurgence due to the ability to create and manipulate such materials (18).

Nanoparticles, which are nanoscale, small particle-like systems with the capacity to operate autonomously in a process, are created by the reduction of particular metal salts. Metals and metal oxides are used to create some of the more efficient nanomaterials (19).

Recently, several methods have been used to modify plant development using metal nanoparticles made of pure metal and metal oxides. These metal nanoparticles have a variety of impacts on various plants, as evidenced by the various plant development indicators, including germination, growth, biomass accumulation, and biochemical activities (21).

Nanomaterials hold great potential for use in nutrition and plant protection because of their distinct optical properties, high surface-to-volume ratio, and size-dependent properties (22).

The impact of nanoparticles on plants ranges from advantageous to detrimental, ranging from overt physiological changes to subtle biochemical alterations. When advantageous, these nanoparticles accelerated germination and growth, which raised the size of the shoot and root. Additionally, the nanoparticles may be harmful to plants by reducing the quality of the seeds, increasing the formation of reactive oxygen species (ROS), and altering the genetic and anatomical characteristics of the plants (19) (22) (21) (23).

Moreover, different types of nanoparticles at various concentrations may cause different reactions that depend on plant species (23).

It is still unknown how nanoparticles accelerate secondary metabolism. One potential mechanism is the generation of reactive oxygen species (ROS) by the nanoparticles, which ultimately results in the activation of secondary metabolism to handle the stress that the ROS have produced. Up-regulating the expression of several genes implicated in the manufacture of secondary metabolites is another method of boosting their production (24) (25).

NPs can enter plants, travel to remote parts of a plant, and then act there because of their small size (26). Using nanoparticles (NPs), which are known to be environmentally friendly, to manage plant diseases may therefore reduce the need for pesticides in general and improve food security (27). Also, nanoparticles can be used to control the release and targeted delivery of nutrients, especially micronutrients, since plants require only a small amount of micronutrients (28) (29). Hence, they increase the possibility of unleashing the potential of sustainable and precision agriculture (29). These beneficial commercial developments have sparked a sharp increase in the production of tailored NPs, making nanotechnologies a quickly evolving subject.

The most common nanomaterials produced globally are zinc oxide (ZnO) and titanium dioxide (TiO2) (30). Because of their ability to block UV light and the visible transparency of their nanoparticle forms, zinc oxide and titanium dioxide are widely used in sunscreens, cosmetics, and bottle coatings (31).

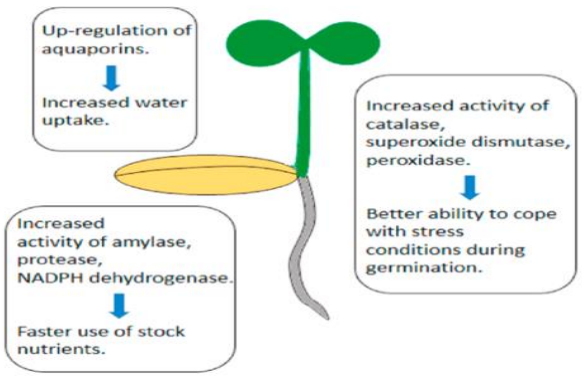

Figure 1.2. Beneficial effect of metal NPs for seed germination and seedling development (32).

1.2.1 Zinc oxide NPs:

ZnO NPs have unique properties like a low-energy bandgap and a piezoelectric action (33). As a result of their significant photochemical activity and high catalytic activity, as well as their distinct antifungal and antibacterial properties, ZnO NPs have a wide range of applications (34).

Moreover, evidence suggests that Beta vulgaris' pathogen activity was reduced by the buildup of ZnO Nanoparticles, which inhibited disease (35).

The impact of ZnO NPs on crop plants has been extensively assessed (36). While numerous studies have demonstrated that ZnO NPs can increase chlorophyll production and photosynthetic activity in both normal and stressful environments (32). The repression of genes involved in chlorophyll synthesis and the design of the photosynthetic system are the primary causes of the toxicity effects of ZnO NPs observed in some plants treated with ZnO NPs (37).

This suppression of chlorophyll biosynthesis results in decreased photosynthetic efficiency in plants. Depending on the plant species and the concentration of ZnO NPs, ZnO NPs can have a variety of effects. (38), in addition to the application method (39).

Furthermore, the efficacy of plants in removing heavy metals from contaminated water can be enhanced by nano-treatment by ZnO NPs at optimum concentration, and this is remarkable (40), 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.

1.2.2 Titanium dioxide NPs:

One of the most widely produced nanomaterials worldwide is TiO_2 NPs, which are significant as a photocatalysts (32) (41), which enhance the light absorption. Also, they are employed in the food industry, cosmetics (sunscreen to block UV-A and UV-B rays), cleaning products, and pigments (41). Because of these many applications, the production of TiO_2 NPs has increased dramatically. It is also noteworthy that the TiO_2 NPs were found to stimulate RuBisCo activity, increase photosynthetic rate, and prevent chloroplasts from aging during lengthy illumination regimes (42).

TiO2 NPs may also have an impact on plant growth by altering hormone levels, in addition, they can influence the amount of phytohormones that promote plant growth, shield plants from cadmium uptake from contaminated soils, and aid in phosphorus uptake (32). Which may be an advantage for TiO_2 NPs as potential candidates for further exploitation in agricultural applications.

TiO2 NPs have a significant anti-fungal activity (43), as well as strong antibacterial properties (39) (43).
As well, the photo-catalytic surface characteristics and direct antibacterial activity of TiO_2 NPs have been implicated in their disease-suppressing effects. Additionally, the antioxidant defense mechanism of Beta vulgaris was activated thanks to TiO_2 NPs, which in turn decreased disease indices (43). And there is considerable evidence that TiO_2 NPs help crops grow and become more resilient to abiotic stressors (45).

All these characteristics of titanium dioxide nanoparticles are the reason for the increasing interest in it, the escalation of its manufacture and production, and the tireless attempts to employ it more and more in agricultural applications.

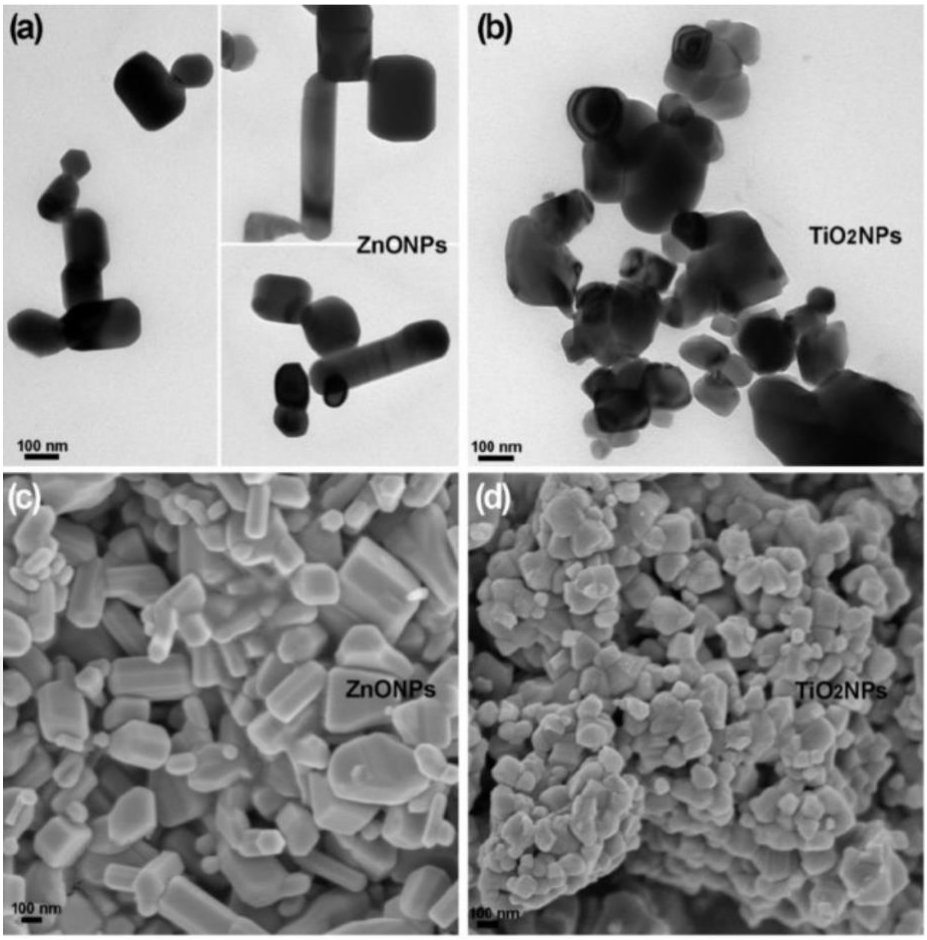

Figure 1.3. SEM ans TEM image of ZnO NPs and TiO_2 NPs: a,b) TEM view of polymorphic ZnO NPs and TiO2NPs with dimensions of 20-200 nm. c,d) SEM view of polymorphic ZnO NPs and TiO2NPs. (46)

1.3 Secondary metabolites in plants:

Secondary metabolites are produced by plants in a variety of ways, the majority of the secondary metabolites are defense mechanisms against herbivores and microbes as well as some secondary metabolites aid plant communication, and protect plants from abiotic stress (47).

Despite the fact that more than 100,000 metabolites are believed to be involved in the plant defense system, secondary metabolite formation comes from millions of years of plant interaction with pathogens (48), They are still being investigated in order to fully comprehend them.

Secondary metabolites from plants have been recognized as significant sources for pharmaceuticals, cosmetics, food additives, and flavorings, in addition

to many commercial products. These bioactive compounds frequently accumulate in plant cells and cultures when those environments are exposed to various stresses, such as excitants or signaling molecules (49).

With the increase in pathogens, which pose a great threat to agricultural crops in the world, there is a need to develop ecologically friendly strategies that harness the formation of plant secondary metabolites that protect plants against numerous pathogens (50). The most significant secondary compounds in sugar beet are betaine and betalain.

1.3.1 Betaine:

Trimethyglycine, oxyneurine, and glycine-betaine are all names for betaine which is also a zwitterionic quaternary ammonium compound, It was first identified in the sugar beet plant, where it naturally occurs and accumulates (13).

A crucial tactic for plant defense and life in harsh environments is metabolic acclimation, which is achieved through the accumulation of osmolytes.

Certain plants produce significant amounts of glycine betaine (betaine) in response to high salinity, cold, and drought, implying that betaine is most apparently involved in the defense of plant cell macro-components like protein complexes and membranes under stressful conditions (51).

Due to its effective role in maintaining important and basic plant functions, it is one of the most studied secondary metabolites.

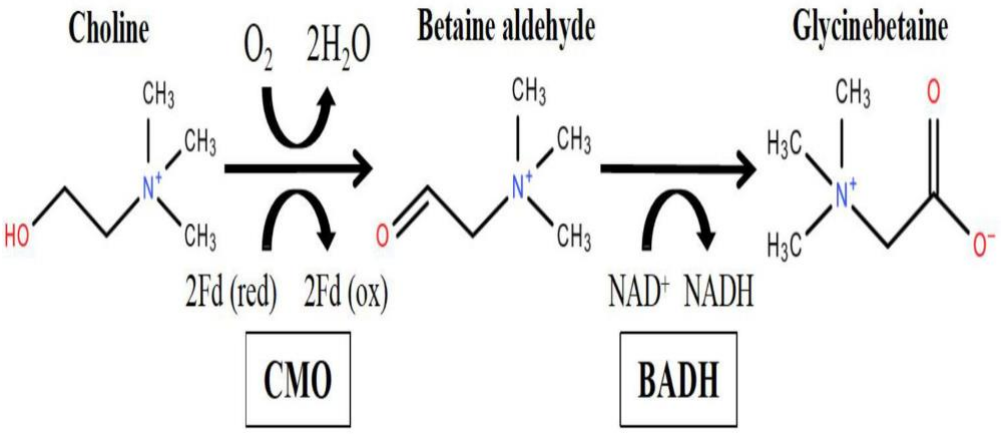

Figure 1.4. Choline is converted into glycinebetaine (GB) in two steps in plants: choline monooxygenase (CMO) catalyzes the first reaction, and betaine aldehyde dehydrogenase (BADH) catalyzes the second (51).

Since sugar beet is a salt-tolerant plant, betaine has the greatest credit for this. Knowing that young leaves accumulate more betaine than mature leaves, betaine in sugar beet molasses has become economically valuable. This compound and the remaining sucrose are purified by chromatographic separation (13).

Betaine is derived from oxidized choline and is highly soluble in water but almost insoluble in most organic solvents. It is also used in the food and cosmetic industries, as well as nutritional supplements. Commercially, betaine is a natural byproduct of the sugar beet industry, and the human body gets nearly all of the betaine it needs from the diet, either from food or from dietary choline (52).

Betaine is a provitamin that acts as a source of methyl groups for methylation reactions (a methyl donor) (53). The main function of betaine is to protect cells from osmotic disruption (53) (54). This means that betaine's most important function as an osmolytic is to protect cells under stress. 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 (54).

Improving salinity tolerance in major crop plants is an urgent issue for the international community due to the rapid growth of the world's population. The fact

that soil salinity inhibits plant growth through osmotic stress and ion-specific toxicity. Here, the importance of osmotic substances such as betaine, which accumulates naturally in some types of plants, including sugar beet (13), is highlighted. This is one of the reasons for the growth of the beet crop and its spread in many countries of the world, and it is constantly being developed and studied.

1.3.2 Betalains:

Betalains are nitrogen-containing vacuolar pigments that are water-soluble (55). They are natural pigments that primarily consist of yellow-orange betaxanthins or red-violet betacyanins, which are betalamic acid derivatives (56). *Beta vulgaris L.* is used in the food companies in 11 different species, including sugar beets, and betalains, which are indole-derived glycosides, cause the characteristic red coloring of their tissues (57).

Although betalains are not widely distributed in the plant world, their properties make them popular in the food industry as a source of their natural red color and as a food additive. It can be used as a natural colorant for many food products or to enhance their appearance (56).

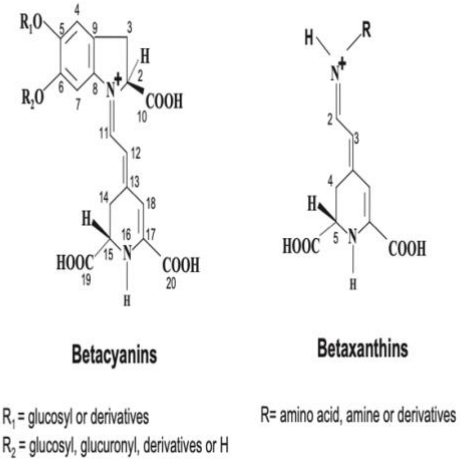

Figure 1.5. Betacyanin and betaxanthin structures, the two main components of betalains (55).

These secondary metabolites are formed by the action of enzymes on the polar aromatic amino acid tyrosine to produce dihydroxyphenylalanine (L-DOPA) and betalamic acid [4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridene-2,6-dicarboxylic acid], the precursor compound for the synthesis of both subgroups of betalains (betacyanin and betaxanthin) (57).

Betalamic acid is a dihydropyridine alternative with a conjugated double bond structure. Betaxanthin is created by combining betalamic acid with amines or amino acids, while betacyanin is created by condensation of cyclo-l-(3, 4-dihydroxylphenylalanine) or its glucosyl derivatives (58) (59). Approximately 78 betalains have been identified in plants from 17 different families (60) (61).

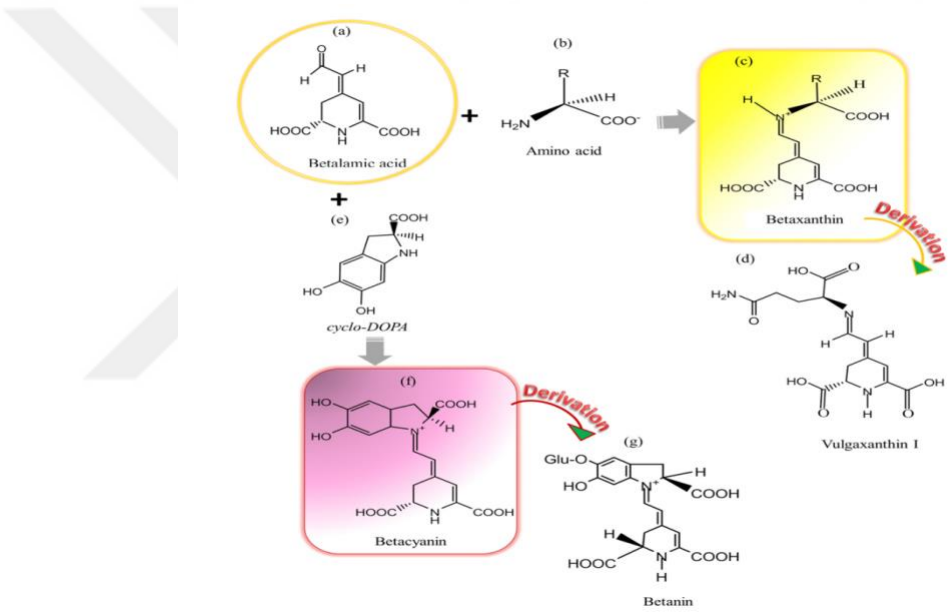

Figure 1.6. The process by which betacyanin and betaxanthin are produced from betalamic acid includes the following steps: (a) Betalamic acid, which is the primary component of betalain; (b) Amino acid, which conjugates with betalamic acid to produce betaxanthin; (c) Yellow color producing betaxanthin; (d) Vulgaxanthin I, a derivative of betaxanthin; (e) cyclo-DOPA, which condenses with betalamic acid to produce betacyanin; (f) Red-violet color producing betacyanin, (g) Betanin, which is a derivative of betacyanin (59).

In addition to being natural colorants, they have properties that enhance safety and health, moreover due to the structure of betalains, they have considerable

antioxidant activity that can outperform ascorbic acid and other molecules with similar effects (61).

A important relationship was discovered between the capacity for antioxidants and red pigment content, but not between antioxidant capacity and yellow pigment content; this shows that betacyanin possesses the main antioxidant capacity (58).

Furthermore, both are non-toxic and non-allergenic, with stable pH levels and an elevated molar extinction coefficients (60).
Although both types of betalain demonstrated different radical scavenging activity, betacyanins had higher antiradical activity than betaxanthin; however, pH had a considerable impact on betalain antiradical activity; betacyanins and betaxanthins both demonstrated greater antiradical activity ratings at neutral and basic pH levels than acidic pH levels (62).

As it is known that free radicals cause many disorders, betalain, with its antioxidant and anti-radical effects, plays an important and healthy role in maintaining life and resisting stresses. Furthermore, betalains have anti-cancer, antiviral (63) (64), anti-inflammatory properties, antibacterial, hepatoprotective, and, as well as gut and immune regulating properties (64).

Overall, natural betalain pigments have the potential to be employed not just as safe additions to provide natural color or antioxidant capabilities to foods, medicines, and cosmetics, but also to promote health and disease prevention. Because the stability of natural dyes in terms of appearance and molecular integrity is critical to delivering the expected biological effect, extrinsic factors influencing their betalain stability include oxygen, temperature, pH, light, and processing and food product chemicals (65).
This point was the subject of study and the interest of several researchers who sought to develop the stability of betalain as a natural dye of high quality, with many benefits. On the other hand, the beneficial properties of betalains, such as their safety, abundance, ease of extraction, accessibility, and biodegradability,

aided in the development and application of these natural pigments as food ingredients (64).

1.3.3 NPs as inducers of secondary metabolites:

Secondary metabolites are well known for their defensive role in plants against abiotic and biotic stressors such as pathogens, pests, herbivores, and predators (50). It is arguably the most amazingly sophisticated defense system in plants. In general, NPs have been shown to be toxic to plants by generating reactive oxygen species (ROS). Considering the relationship between ROS and secondary signaling reporters, leading to transcriptional regulation of secondary metabolites, it is evident that ROS generated by nanoparticles will act as a catalyst for the induction of secondary metabolism in plants (66).

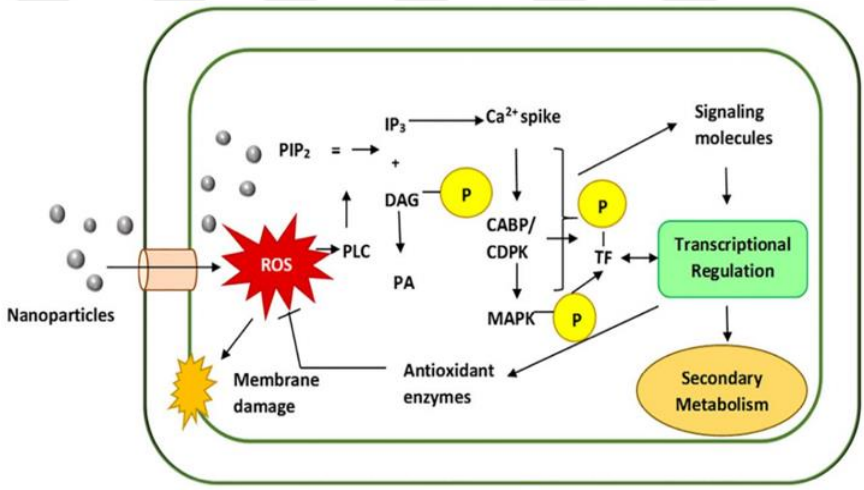

Figure 1.7. Schematic illustrating putative processes involved in nanoparticle-mediated plant metabolic control (67). This is accomplished by reprogramming secondary metabolite transcription, in which antioxidant activation of enzymatic and non-enzymatic mechanisms such as secondary metabolism may scavenge ROS and protect cells from oxidative damage (59).

For a long time, scientists have known that nanoparticles can cause abiotic stress and toxic effects in plants. However, they are only recently being recognized as molecular pharming tools for eliciting beneficial secondary metabolites in plants both in vivo and in vitro, as well as agents for harvesting secondary metabolites from plant cells as comparators (67) (68). These two applications have enormous

commercial potential. For example, sugar beet has enhanced betalain pigment expression under stress and specific culture conditions (69).

Large dose response studies must be carried out to determine the ideal concentration for each NP-plant system in order to maximize secondary metabolite yield while minimizing toxic effects on the plant, the product's consumer, and the environment. Since the function of nanoparticles as secondary metabolite elicitors is highly dependent on their chemical and size, mineral composition, and occasionally shape, as well as the concentration of application (67).

1.4 Photosynthesis:

The most important and fundamental physiological function in plants is photosynthesis. It requires using the chloroplasts to transform light energy into chemical energy and storing it in an environment with sugar bonds (21). Photosynthesis is a sequence of complicated events in which carbon dioxide (CO2) and water (H2O) mix together to make glucose and fructose in the sugar beet plant, which absorbs solar energy. Two reactions occur: the light reaction and the dark reaction. Chlorophyll absorbs light energy in the light reaction, producing monosaccharides that then accumulate in plant molecules, and the energy in the dark reaction is used to produce monosaccharides by reacting carbon dioxide with water (8).

In other words, sucrose, the main reason for sugar beet cultivation and popularity, results from photosynthesis as a primary metabolite.

The photosynthetic pigments chlorophylls and carotenoids, which are isoprenoid plant lipids, are also referred to as prenyl pigments (70). They are only found naturally in plants; chlorophyll and carotenoids are common organic food components (71).

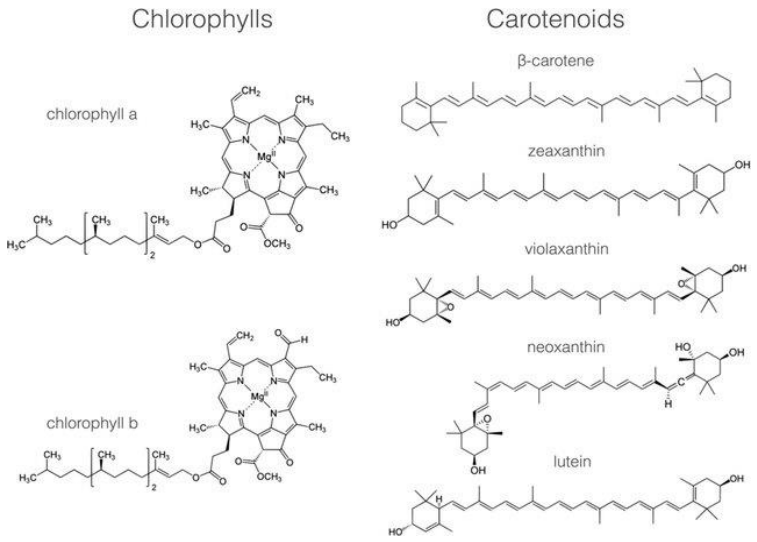

Figure 1.8. Plant photosynthetic pigments and the chemical structures of the chlorophylls and carotenoids pigments, are found in the thylakoid membrane of the chloroplast, where photosynthesis light reactions occur (71).

Photosynthesis is a very vulnerable biological activity under abiotic stressors, and NPs have been demonstrated to protect the process and boost photosynthetic efficiency by lowering oxidative and osmotic stress (72). However, it is important to note that the effects of NPs on photosynthesis vary between plants and even between species (73). The effects also differ depending on the type of nanoparticles used to treat the plant (21).

Moreover, many studies showed that different application methods of ZnO NPs can enhance chlorophyll biosynthesis and photosynthetic activity under both normal conditions and stress conditions, TiO_2 NPs also have the same ability to improve chlorophyll biosynthesis, increase photosynthesis, and stimulation of nitrogen metabolism (32).

Photosynthesis is one of the most important processes on Earth and is linked to other most important processes in life. It can be said that life in general is associated with this process. Many scholars have studied its details in depth and everything that affects it positively or negatively. Of course, improving its efficiency is an ongoing work that has priority in many studies.

1.5 Tissue culture:

Tissue culture is fundamentally used to prepare for transformation, which is now fairly common in large breeding companies, sugar beet tissue culture techniques include clonal propagation via meristem culture, regeneration from callus tissues derived from nearly all plant organs, and somatic embryogenesis (74). It is primarily determined by the genotype of the plant, but it is generally achievable by modifying the culture media conditions and, in some cases, the source culture tissue (75).

Meaning that plant tissue culture is intrinsic to plant biology and is essential for conservation, mass reproduction, genetic manipulation, bioactive compound production, and plant improvement.

1.5.1 Tissue culture with NPs:

In recent years, the application of NPs to tissue culture has led to the successful removal of microbial contaminants from excised plants, in addition to demonstrating their role in callus induction, organogenesis, somatic embryogenesis, somatic differentiation, epigenetic transformation, and secondary metabolite yield. However, the effectiveness of NPs in removing microbiological contaminants in plant tissue cultures can be determined by their dimensions, size, distribution, and type (76).

Since bacterial contamination is a serious problem in tissue culture and has many negative effects, the properties of nanoparticles as antibacterials are very useful in this regard.

2 AIM AND SCOPE OF THE STUDY:

This thesis focuses on discussing the effect of nanoparticles of zinc oxide and titanium dioxide on the sugar beet plant, which is one of the most important agricultural crops in the world and in Turkey in particular, in several aspects, including:

1. Seed germination process and comparison of morphological changes resulting from the applied nanoparticles.
2. Effect on photosynthesis through estimation of chlorophyll and carotenoids.
3. Influence the production of the secondary metabolites betaine and betalain, which are two of the most significant secondary metabolites found in sugar beet plants.

3 MATERIALS AND METHODS

3.1 Plant material:

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

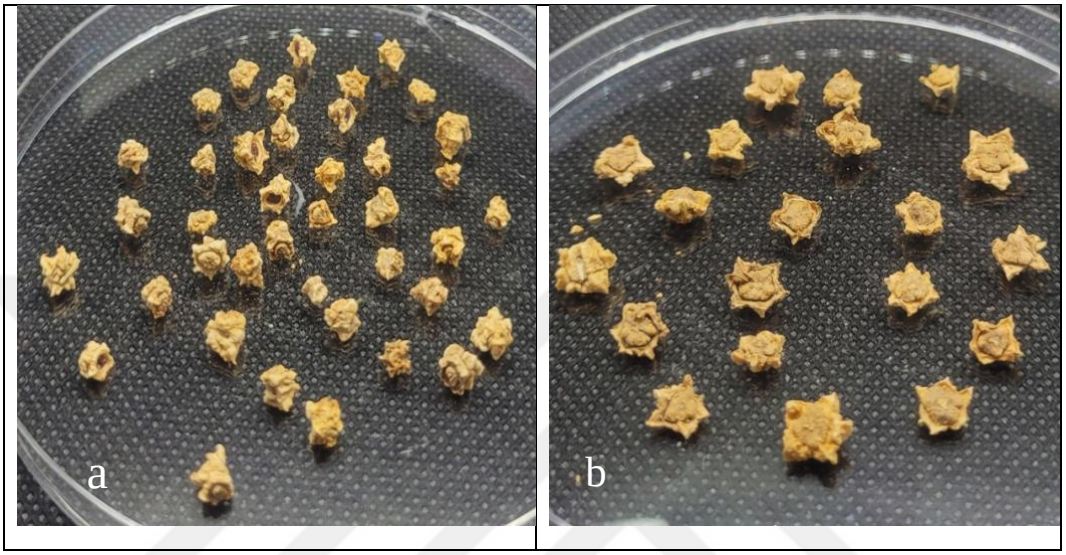

Figure 3.1. Sugar beet seeds: a) SG2020 & b) SG833.

3.2 Germination condition:

Two genotypes of sugar beet seeds, SG833 and SG2020, were used for the experiments. The following sterilization procedure was used:

- After surface sterilizing the seeds of two sugar beet genotypes with 70% ethanol for 5 minutes, they were treated with 70% sodium hypochlorite and Tween 20 (0,5ml/100ml) for 60 minutes.
- The seeds were then rinsed with sterile, distilled water and washed again until the water was clear.
- After that, soak in sterile distilled water at 25 degrees Celsius overnight..
- After removing the water, the seeds were washed with a 5% PPM solution (plant preservation mixture, Plant Cell Technology Inc., WA, USA).

- After sterilization, sugar beet seeds were kept in nanoparticle solution for 24 hours,with the solution containing different concentrations of ZnO and TiO_2 (0, 5, 15, 25), while control seeds were kept in sterile distilled water for 24 hours.
- Afterward, these seeds were germinated in vitro in MS nutrient medium (77), containing the same concentrations of the relevant NPs, in sterile plastic Petri dishes 100 x 15 and at least 10 dishes for each treatment.
- The seeds were kept at 23 ± 1 ^{o}C in the dark for 5 days before being switched to a 16-hour light cycle with an 8-hour dark photoperiod (provided by cool-white, fluorescent light) at a relative humidity of 55-60% until they were 7 days old after germination.
- These 7 days old seedlings were then transferred to high-sterile glass jars that contained MS nutrient medium with NPs in the same concentrations.

3.3 Nanoparticles stock solutions:

The titanium IV oxide (TiO_2, Cat # 700347) and zinc oxide (ZnO, Cat # 721077) NPs, both of which were purchased from (Sigma - Aldrich).

The ZnO and TiO_2 NPs, were prepared separately in stock solutions at a concentration of 25 mg/mL and, after dispersion with sonication, diluted at different concentrations (0, 5, 15, and 25) mg/ml for the experiments.

3.4 Preserving plant samples:

Sugar beet seedlings were harvested 4 weeks after germination. Harvested seedlings were kept in aluminum foil and freeze with liquid N2, then preserved at -80 °C.

3.5 Estimation of growth and development of plants with different parameters:

3.5.1 Germination rate:

The percentage of germination was recorded from the first day of germination, and on a daily basis, updates were added. And using the Excel program for calculations related to the germination rate. The germination rate was calculated using the following equation:

% germination rate = number of germinated seeds / total seeds multiplied by 100.

3.5.2 Analysis phenotypic effect of plants:

The morphological effect of different NPs treatments on 4-week-old sugar beet plants was compared to the control group. This was done by measuring the root length and hypocotyl length of each plant separately using ImageJ software.

3.5.3 Measurement of chlorophylls and carotenoids:

Chlorophyll (Chl) a, (Chl) b and carotenoids were measured and determined according to Lichtenthaler and Welburn (1983) (78). Briefly, 4-week-old whole plants were collected and extracted by grinding the samples to a fine powder and measuring 100 mg of the sample, then transferring it to a 2 mL Eppendorf tube supplemented with 1 mL of 96% ethanol and homogenizing for 10 min at 4 °C. Then the samples were kept overnight at a temperature of -20 °C .An ultrasonification device was used, and after that, the samples were placed in a centrifuge at a temperature of -4 °C for 5 minutes. The absorbances were measured by a UV spectrophotometer at 479, 649, and 665 nm. Using absorbance values in Excel, calculations were made to determine the value of each Chl a, each Chl b, and carotenoids for both genotypes of sugar beet.

$C_a = 13.95 \times A_{665} - 6.88 \times A_{649}$

$C_b = 24.96 \times A_{646} - 7.32 \times A_{665}$

$C = 1000 \times A_{470} - 2.05C_a - 114.8C_b / 229$

3.5.4 Estimation of the secondary metabolite betaine:

The experiment was carried out according to the following protocol (79), with modifications:

- At first, 250 mg of samples were measured after grinding well and added to a tube with 5 ml of distilled water.
- Then the samples were incubated for 24 hours at a temperature of 25 °C.
- The next day, the samples were placed in a centrifuge at room temperature for 10 minutes. Then 4 mL of the solutions were transferred to another tubes, and 4 mL of sulfuric acid H2SO4 (cat #1.00731.2500, from Merck KGaA), 1:1, 2N, were added.
- After 1 hour of cooling in an ice bath, 100 ml of potassium iodate-iodin KI-I (potassium iodide (cat # 7681) and iodine (cat # 7553562) from Merck were added to each tube and gently vortexed.
- After that, the samples were kept at a temperature of 0-4 °C for 16 hours. After 16 hours, betaine crystals will have formed.

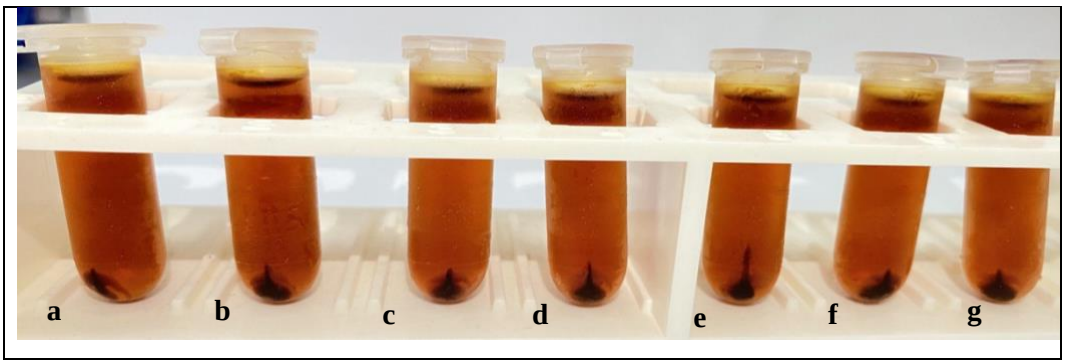

Figure 3.2. The betaine crystals formed in genotype SG2020 are: a) control, b) TiO_2 5 mg/ml, c) TiO_2 15 mg/ml, d) TiO_2 25 mg/ml, e) ZnO 5 mg/ml, f) ZnO 15 mg/ml, g) ZnO 25 mg/ml.

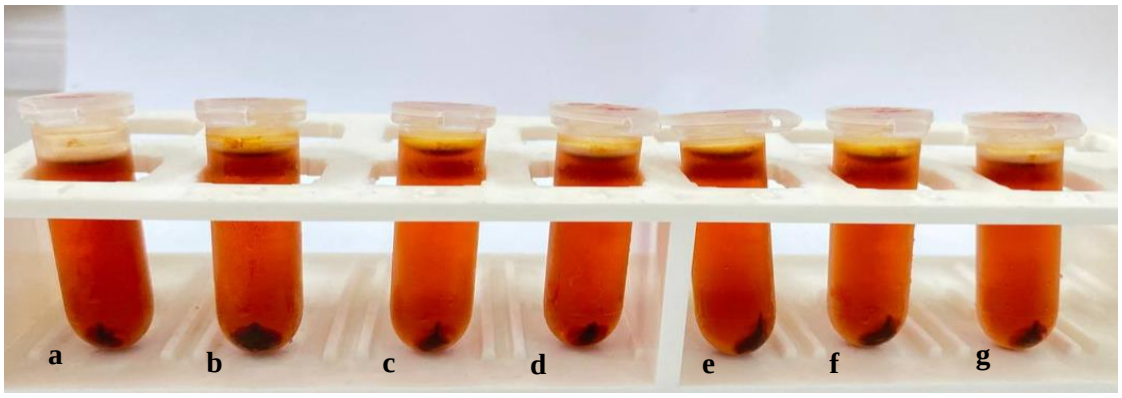

Figure 3.3. The betaine crystals formed in genotype SG833 are: a) Control, b) TiO_2 5 mg/ml, c) TiO_2 15 mg/ml, d) TiO_2 25 mg/ml, e) ZnO 5 mg/ml, f) ZnO 15 mg/ml, g) ZnO 25 mg/ml.

- The tubes are centrifuged for 15 minutes at 0 °C, the liquid is removed, and the betaine crystals are preserved.
- Betaine crystals are dissolved in 4.5 mL of 1,2 Dichloroethane (cat # 03530 was purchased from Fluka Analytical).
- After that, the tubes were incubated for two hours at room temperature. Finally, the absorption is measured at a wavelength of 225 nm.
- All data were transferred to an Excel file, and calculations were made to obtain the concentration of betaine in each concentration for both genotypes of sugar beet.
- The values were calculated as percentages compared to the control for both genotypes of sugar beet.

3.5.5 Estimation of the secondary metabolite betalain:

The protocol that was used (80), it is as follows:

- 500 mg of finely ground samples were taken, and 1.5 ml of citric acid (cat# 77929 from Sigma) was added to it, which was prepared from 1.5 g of citric acid with 50% ethanol.
- The samples were then gently vortexed and homogenized for 15 minutes in an ice bath.
- Samples were incubated at 52 °C, for 50 minutes.

- Immediately after that, ultrasonics were used to homogenize the samples for a period of 5 minutes, then the samples were placed in a centrifuge for 15 minutes at room tempreture, and the supernatant was transferred to a 2 ml Eppendorf tube.
- Then, absorption readings were taken from the UV-Spectrophotometer, at wavelength 538, 476 and 600 nm.
- Use the data in Excel to be analyzed and the calculations to estimate betanin and Vulgaxanthin I in each concentration for both genotypes of sugar beet.

$$A_{Betanin} = 1.095 \times (A538 - A600)$$

$$A_{Impurities} = A538 - A_{Betanin}$$

$$A_{Vulgaxanthin\ I} = A476 - A_{Impurities} - A_{Betanin} / 3.1$$

$$c_{Betanin} = 25 \times A_{Betanin} / 1120$$

$$c_{Vulgaxanthin}\ I = 25 \times A_{\ Vulgaxanthin\ I} / 750$$

3.6 Statistical analysis:

The findings from three independent experiments were averaged and standard deviations were reported.

Statistical analysis was performed using one way ANOVA , which was followed a Tukey post hoc test, with $p < 0.05$ and $p < 0.01$ were considered significant.

4 RESULTS

4.1 Seed germination analysis:

It was clearly shown the difference in germination between the two plant species, where the multigerm (SG2020) genotype had a higher germination rate than the monogerm genotype (SG833), as shown in figure 4.1.

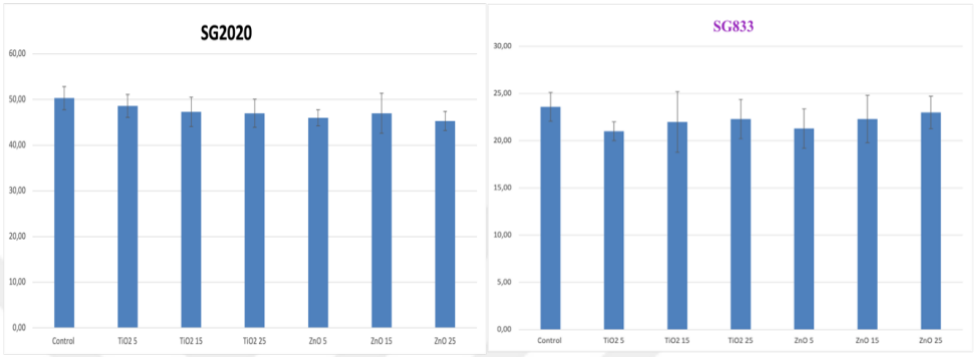

Figure 4.1. The effect of TiO_2 and ZnO Nps on (5, 15, 25) mg/ml concentrations and the control on the seed germination in both genotypes SG2020 and SG833.

In the SG2020 genotype, zinc oxide and titanium dioxide showed a capable effect on the germination process at high germination rates similar and close to the control group, as shown in the figure 4.2. Table 4.1 also contains a statistical analysis.

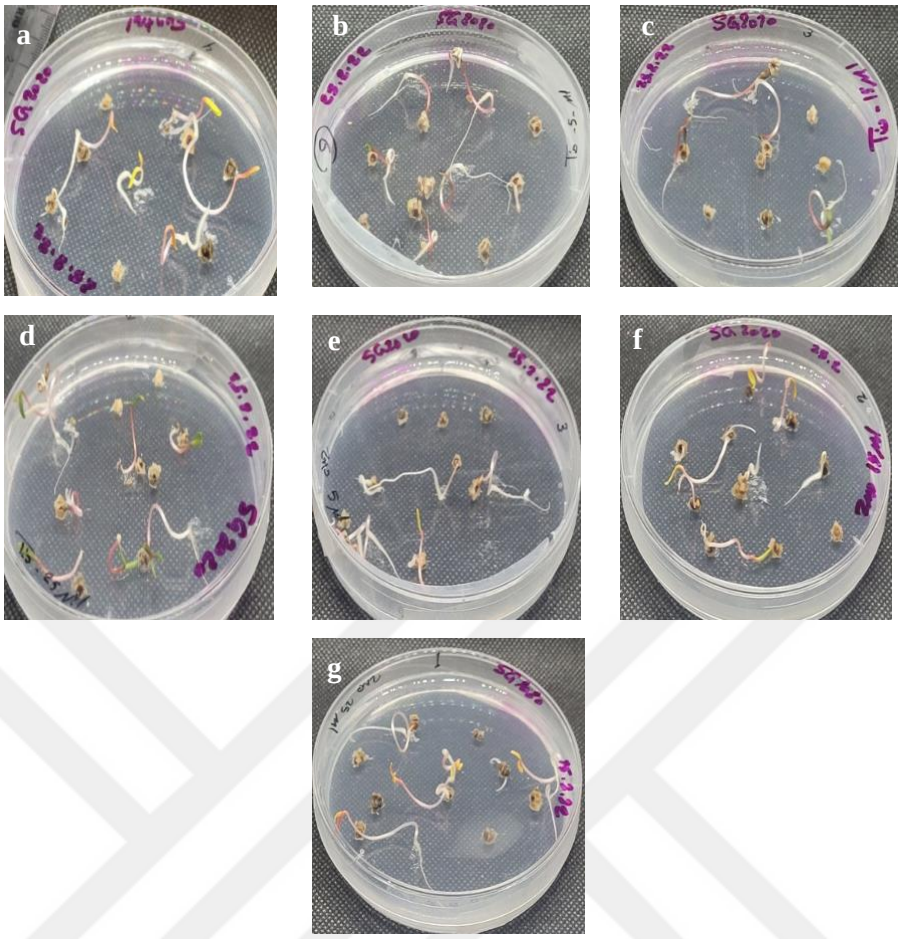

Figure 4.2. SG2020 seeds germination, 5 days after cultivation in (a) MS medium for control, (b) MS medium with TiO_2 NPs 5mg/ml, (c) with TiO_2 NPs 15 mg/ml, (d) with TiO_2 NPs 25 mg/ml, and (e) MS medium with ZnO 5 NPs mg/ml, (f) with ZnO NPs 15 mg/ml, (g) with ZnO NPs 25 mg/ml).

Table 4.1. P value of seeds germination for SG2020 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.5444	0.7655	0.3965	0.9000	0.8391	0.9000
TiO_2 5		0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.9000	0.9000	0.9000	0.9000
TiO_2 25				0.7654	0.7654	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

The TiO_2 and ZnO nanoparticles had a similar effect on SG833 seed germination compared to the control group, which was characterized by late germination and less than the SG2020; in other words, the effect of these nanoparticles on SG833 seeds is similar to the control in terms of germination rates, as shown in figure 4.2 and the statistical analysis shows that there is no significant difference, in table 4.2. Overall, the effect of TiO_2 and ZnO nanoparticles did not cause any difference on the process of seed germination in the two genotypes (SG2020 and SG833).

While the treatment with nanoparticles showed a clear effect as an antibacterial and decreased contamination rates compared to the control, zinc oxide had the greatest effect compared to titanium dioxide.

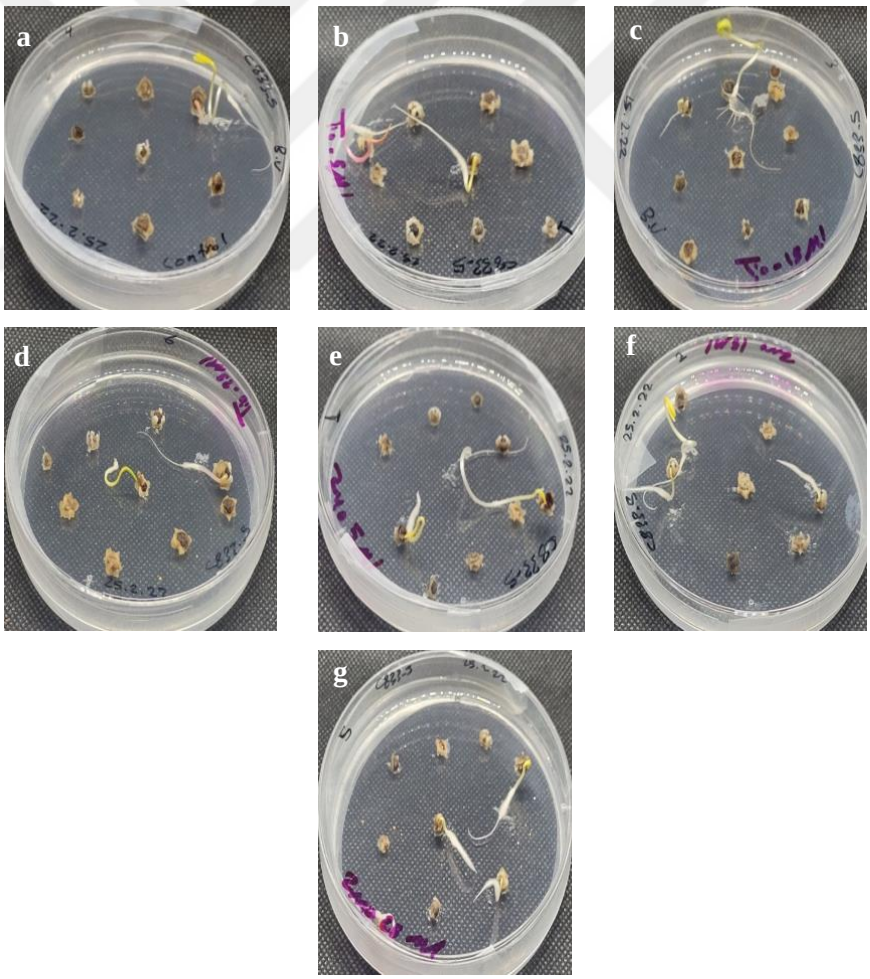

Figure 4.3. SG833 seeds germination, 5 days after cultivation in (a) MS medium for control, (b) MS medium with TiO_2 NPs 5mg/ml, (c) with TiO_2 NPs 15 mg/ml,

(d) with TiO_2 NPs 25 mg/ml, and (e) MS medium with ZnO 5 NPs mg/ml, (f) with ZnO 15 NPs mg/ml, (g) with ZnO 25 NPs mg/ml).

Table 4.2. P value of seeds germination for SG833genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.7000	0.8003	0.9000	0.8003	0.9000	0.9000
TiO_2 5		0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.9000	0.9000	0.9000	0.9000
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

4.2 Comparing the phenotypic effect of plants:

The effect of TiO_2 and ZnO nanoparticles did not differ in all treatments applied to the morphology of 4-week-old sugar beet plants in terms of root length and hypocotyl compared to the control plant group. Compared to control plants, the results were very close in the two genotypes (SG2020 and SG833).

as shown in figures 4.3 and 4.4, as well as the statistical analysis in tables 4.3, 4.4, 4.5, and 4.6. Even in terms of the number, length, and width of leaves, the plants of the two genotypes that were exposed to TiO_2 and ZnO NPs treatments at all concentrations were the same as the control plants.

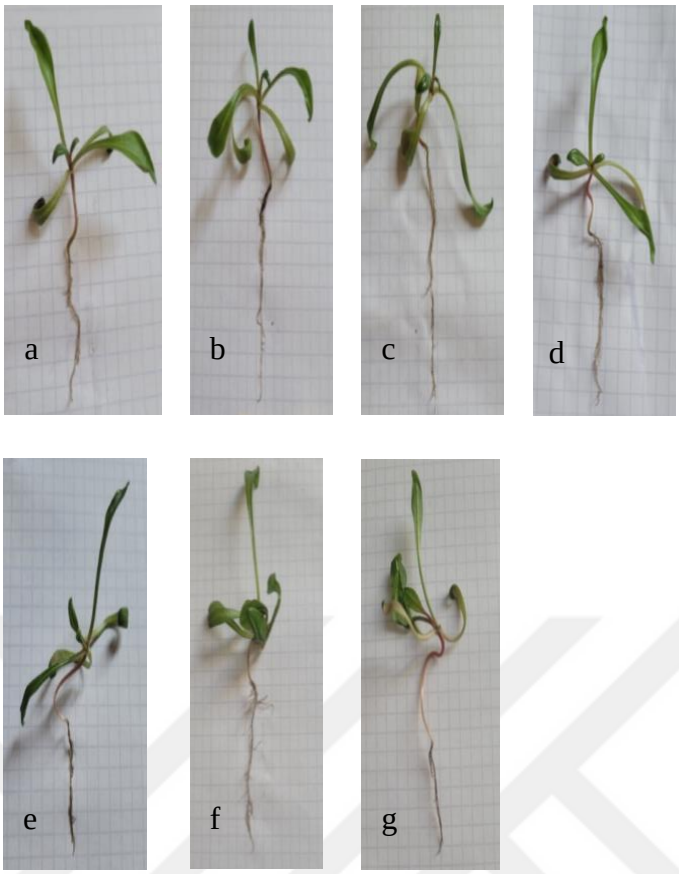

Figure 4.4. Comparing between the Plants in 4 weeks old of SG2020: a) control plant, b) A plant exposed to TiO_2 NPs 5mg/ml, c) A plant exposed to TiO_2 NPs 15 mg/ml, d) A plant exposed to TiO_2 NPs 25 mg/ml, e) A plant exposed to ZnO 5 NPs mg/ml, f) A plant exposed to ZnO 15 NPs mg/ml and g) A plant exposed to ZnO 25 NPs mg/ml).

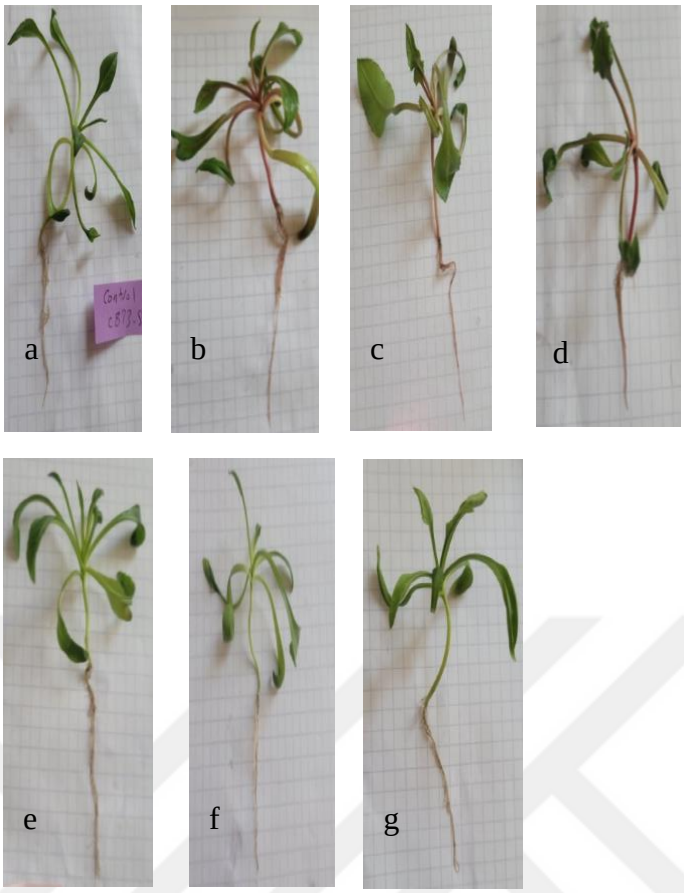

Figure 4.5. Comparing between the Plants in 4 weeks old of SG833: a) control plant, b) A plant exposed to TiO_2 NPs 5mg/ml, c) A plant exposed to TiO_2 NPs 15 mg/ml, d) A plant exposed to TiO_2 NPs 25 mg/ml, e) A plant exposed to ZnO 5 NPs mg/ml, f) A plant exposed to ZnO 15 NPs mg/ml and g) A plant exposed to ZnO 25 NPs mg/ml).

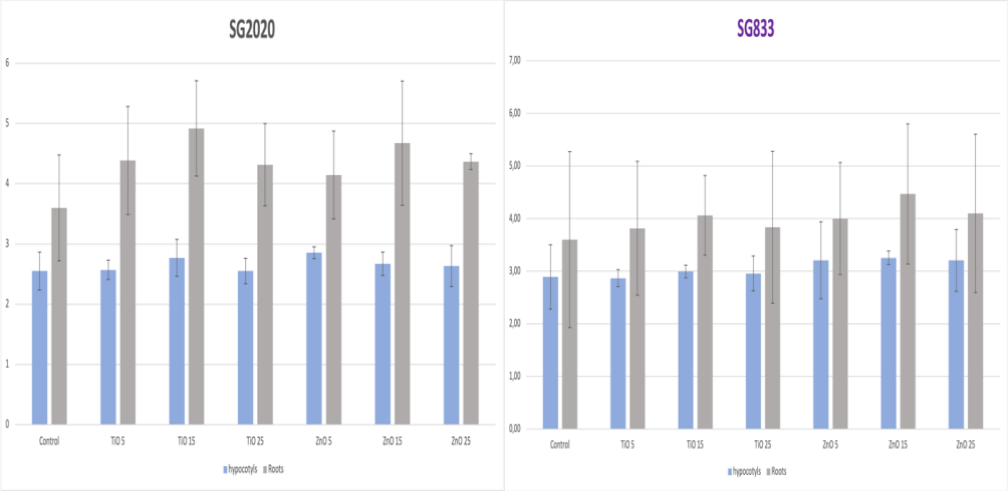

Figure 4.6. The effect of TiO_2 and ZnO NPs on the plant phenotype (roots length and hypocotyls length) in the 4 weeks plant old of SG2020 and SG833 genotypes.

Table 4.3. P value of roots length for SG2020 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.8573	0.4231	0.9000	0.9000	0.6212	0.8732
TiO_2 5		0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.9000	0.8724	0.9000	0.9000
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

Table 4.4. P value of hypocotyls length for SG2020 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 5		0.9000	0.9000	0.8208	0.9000	0.9000
TiO_2 15			0.9000	0.9000	0.9000	0.9000
TiO_2 25				0.7458	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

Table 4.5. P value of roots length for SG833 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.9000	0.9000	0.8922	0.9000
TiO_2 5		0.9000	0.9000	0.8208	0.9000	0.9000
TiO_2 15			0.9000	0.9000	0.9000	0.9000
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

Table 4.6. P value of hypocotyls length for SG833 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 5		0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.9000	0.9000	0.9000	0.9000
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

4.3 Determination of chlorophyll a and b:

Compared to control plant groups, both types of TiO_2 and ZnO nanoparticles and all concentrations applied to plants in both genotypes (SG2020 and SG833) had an improvement in chlorophyll a and b production, as shown in figure 4.5, but through statistical analysis these results were not valuable, as seen in tables 4.7, 4.8, 4.9, and 4.10; therefore it seems that photosynthesis, mainly represented by chlorophyll, was equally effective among all plant groups.

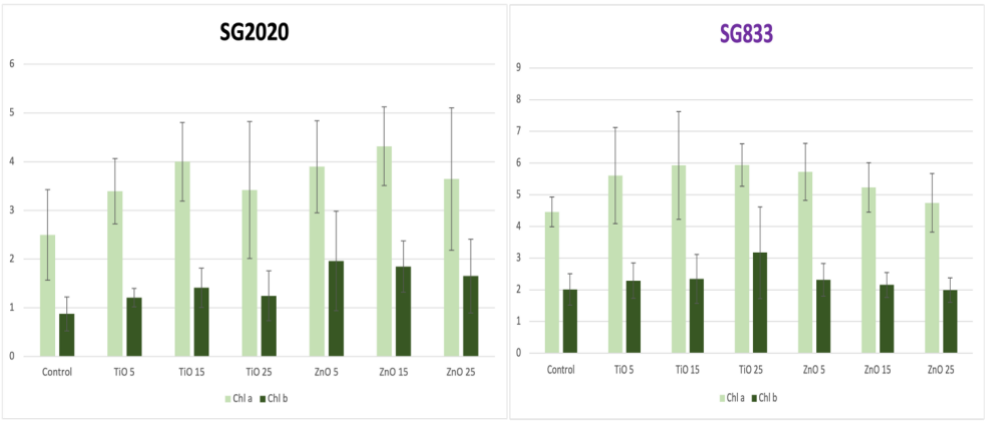

Figure 4.7. Effect of TiO_2 and ZnO NPs at (0, 5, 15, and 25) mg/mL concentrations on the contect of both chlorophyll a and b in both SG2020 and SG833.

Table 4.7. P value of Chlorophyll a for SG2020 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.88412	0.41950	0.1868	0.4970	0.2183	0.1240
TiO_2 5		0.9000	0.7430	0.9000	0.6100	0.6100
TiO_2 15			0.9000	0.9000	0.9000	0.9000
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

Table 4.8. P value of Chlorophyll b for SG2020 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.8677	0.8982	0.2481	0.3543	0.5836
TiO_2 5		0.9000	0.9000	0.6168	0.7480	0.9000
TiO_2 15			0.9000	0.8565	0.9000	0.9000
TiO_2 25				0.8260	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

Table 4.9. P value of Chlorophyll a for SG833 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.7308	0.0640	0.5069	0.6507	0.8922	0.9000
TiO_2 5		0.5623	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.7862	0.6424	0.3168360	0.1285
TiO_2 25				0.9000	0.9000	0.7203
ZnO 5					0.9000	0.8641
ZnO 15						0.9000

Table 4.10. P value of Chlorophyll b for SG833 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.3495	0.9000	0.8922	0.9000
TiO_2 5		0.9000	0.6207	0.8208	0.9000	0.9000
$TiO_2$15			0.6734	0.9000	0.9000	0.9000
TiO_2 25				0.6507	0.4900	0.3281
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

4.4 Determination of carotenoids:

All groups of plants, with all treatments and concentrations, had an effect on carotenoids equal to the concentration of carotenoids in the control groups for both genotypes of sugar beet plants, as shown by figure 4.8, and by the statistical analysis in tables 4.11 and 4.12. Which means again that the photosynthesis process was equally efficient in all groups.

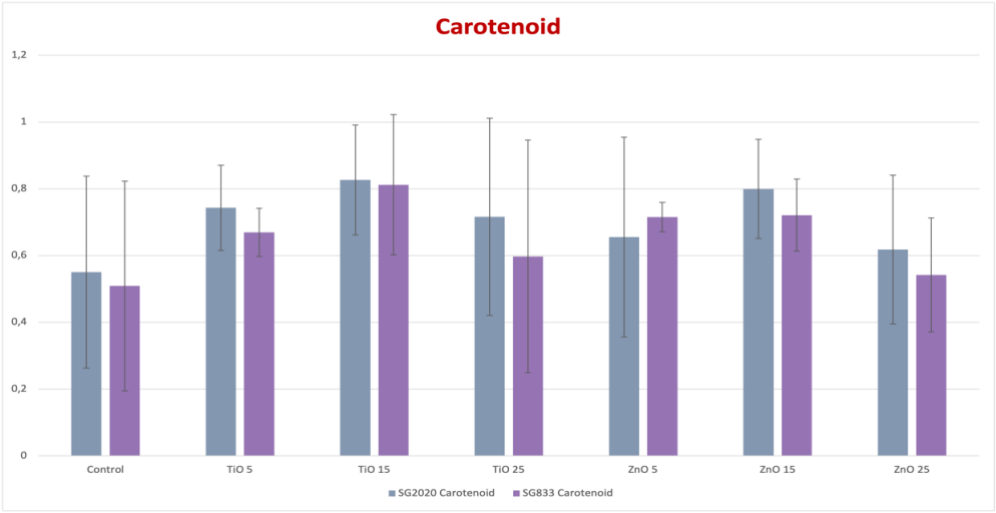

Figure 4.8. Effect of TiO_2 and ZnO NPs at (0, 5, 15, and 25) mg/mL concentrations on carotenoid content in both SG2020 and SG833.

Table 4.11. P value of carotenoid for SG2020 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.7520	0.9000	0.9000	0.8343	0.9000
TiO_2 5		0.9000	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.9000	0.9000	0.9000	0.8738
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15						0.9000

Table 4.12. P value of carotenoid for SG833 genotype using post-hoc Tukey HSD test ($p< 0.05$ *, $p< 0.01$ ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.4722	0.9000	0.9000	0.8922	0.9000
TiO_2 5		0.8612	0.9000	0.9000	0.9000	0.9000
TiO_2 15			0.4792	0.7722	0.7800	0.6210
TiO_2 25				0.9000	0.9000	0.9000
ZnO 5					0.9000	0.9000
ZnO 15					0.9000	0.9000

4.5 Estimation of betalain (betanin and vulgaxanthin I):

The effect of nanoparticles on betanin (a component of betalain derived from betacyanin) in the genotype SG833 plants did not show a clear difference from its concentration in control plants, except for the plants that were exposed to 25 mg/ml of ZnO nanoparticles, which showed a clear increase in the concentration of betanin, as shown in figure 4.9. It is also consistent with the statistical analysis, which revealed a significant difference in the concentration values of ZnO NPs at 25 mg/mL, as shown in table 4.13. As for Vulgaxanthin I, which is derived from betaxanthin, the other component of betalain, the effect of TiO_2 and ZnO nanoparticles on plants showed a significant improvement only in plants that were exposed to nanoparticles of TiO_2 at a concentration of 15-25 mg / ml compared to control plants, as shown in figure 4.9, and the statistical analysis gave a positive difference for these two concentrations, as shown in table 4.14.

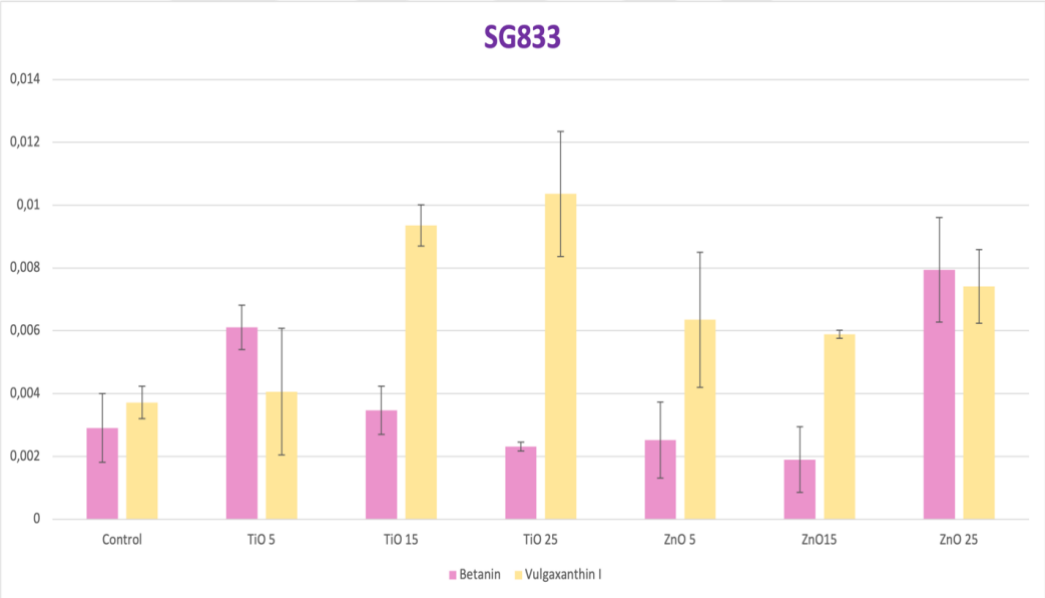

Figure 4.9. Effect of TiO_2 and ZnO NPs at (0, 5, 15, and 25) mg/mL concentrations on the content of both betanin and betaxanthin I in the SG833.

Table 4.13. P value of betanin for SG833 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.9000	0.9000	0.8343	0.0187 *
TiO_2 5		0.9000	0.9000	0.9000	0.9000	0.0111 *
TiO_2 15			0.9000	0.9000	0.9000	0.0259 *
TiO_2 25				0.9000	0.9000	0.0080 **
ZnO 5					0.9000	0.0107 *
ZnO 15						0.0044 **

Table 4.14. P value of vulgaxanthin I for SG833 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG833	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.0027**	0.0010**	0.3309	0.5352	0.0790
TiO_2 5		0.0046**	0.0015**	0.4818	0.6899	0.1302
TiO_2 15			0.9000	0.1488	0.0761	0.5240
TiO_2 25				0.0495*	0.0242*	0.2257
ZnO 5					0.9000	0.9000
ZnO 15						0,8207

Genotype SG2020 plants that were exposed to TiO_2 and ZnO nanoparticles showed no difference in betanin production compared to control plants, as shown in figure 4.10, and in the statistical analysis table 4.15. As for Vulgaxanthin I, it showed a positive response and improvement in plants exposed to ZnO nanoparticles at concentrations of 15 and 25 compared to control plants, as shown in figure 4.10. Furthermore, the statistical analysis in table 4.16 revealed a significant difference in the values of these two concentrations.

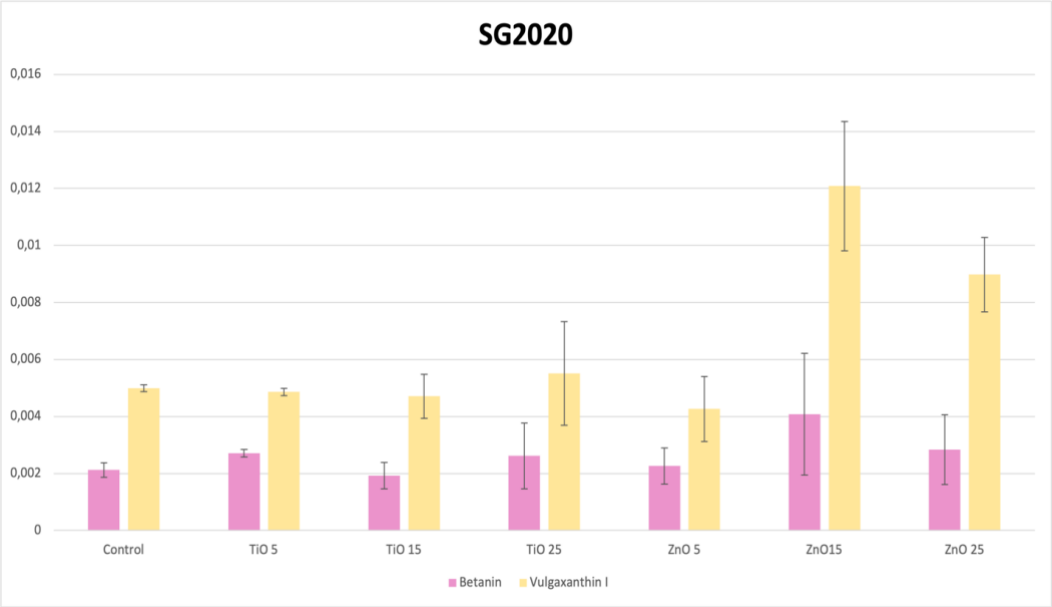

Figure 4.10. Effect of TiO_2 and ZnO NPs at (0, 5, 15, and 25) mg/mL concentrations on the content of both betanin and betaxanthin I in the SG2020.

Table 4.15. P value of betanin for SG-2020 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.9000	0.9000	0.3426	0.9000
TiO_2 5		0.9000	0.9000	0.9000	0.6882	0.9000
TiO_2 15			0.9000	0.9000	0.2476	0.8738
TiO_2 25				0.9000	0.6330	0.9000
ZnO 5					0.4210	0.9000
ZnO 15						0.7648

Table 4.16. P value of vulgaxanthin I for SG2020 genotype using post-hoc Tukey HSD test (p< 0.05 *, p< 0.01 ** statistically different).

SG2020	TiO_2 5	TiO_2 15	TiO_2 25	ZnO 5	ZnO 15	ZnO 25
Control	0.9000	0.9000	0.9000	0.9000	0.0010 **	0.0293 *
TiO_2 5		0.9000	0.9000	0.9000	0.0010 **	0.0234 *
TiO_2 15			0.9000	0.9000	0.0010 **	0.0181*
TiO_2 25				0.9000	0.0010 **	0.0688
ZnO 5					0.0010 **	0.0085 **
ZnO 15						0.1238

4.6 Estimation of betaine:

In the SG2020 genotype, an increased concentration of betaine was observed in plants exposed to TiO_2 NPs and ZnO NPs compared to the control. As shown in Figure 4.11, 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, it reached the highest concentration at Zn 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.

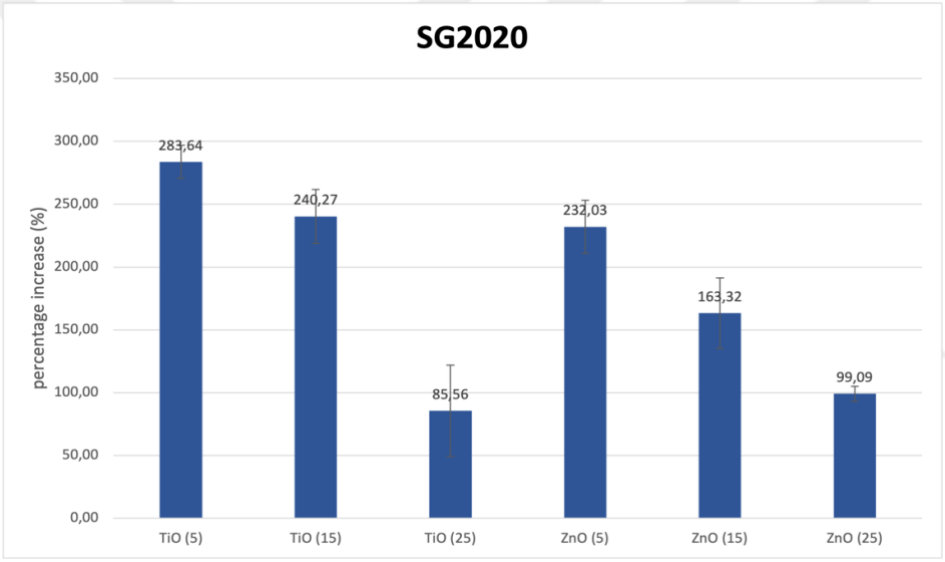

Figure 4.11. Effect of TiO_2 and ZnO NPs at (0, 5, 15 and 25) mg/mL concentrations on betaine content in the genotype SG2020.

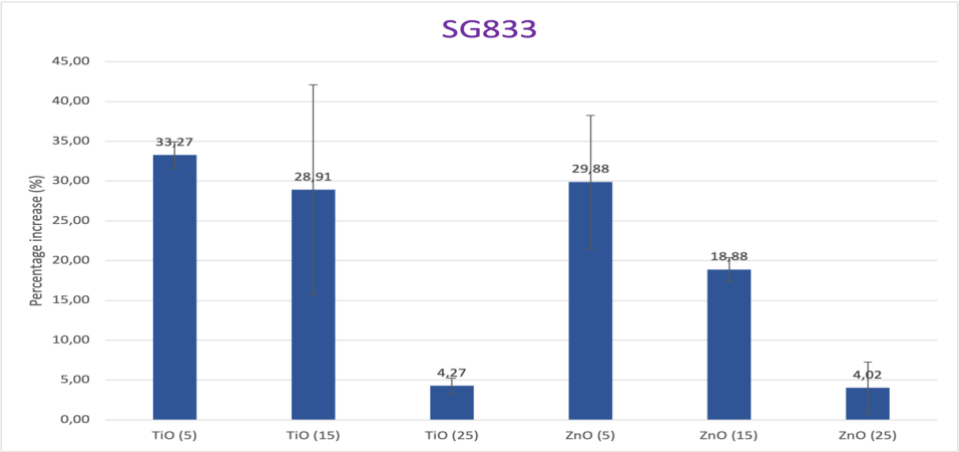

Figure 4.12. Effect of TiO_2 and ZnO NPs at (0, 5, 15, and 25) mg/mL concentrations on betaine content in the genotype SG833.

Likewise, the results for the SG833 genotype were consistent with previous results for SG2020. As shown in Figure 4.12, the highest concentration of betaine at a concentration of 5 mg/ml of TiO_2 NPs reached 33.27% and gradually decreased at a concentration of 15 mg/ml to 28,91%, then it was the lowest value 4.27% at a concentration of 25 mg/ml of TiO_2 NPs, with respect to control plants. Regarding ZnO NPs, the content of betaine according to the control plants ranged from 29.88%, 18.8%, and 4.02% in plants at concentrations of 5, 15, and 25 mg/mL, respectively.

5 DISCUSSION

The most important results of this research, which studied the effect of two types of nanoparticles, TiO_2 and ZnO, at concentrations of (0, 5, 15, and 25) on two types of genotypes of the sugar beet plant, one of which is monogerm (SG833) and the other is multigerm (SG2020), on several parameters: seed germination, morphogenesis (roots and hypocotyls), total chlorophyll and carotenoids, and the secondary metabolites betaine and betalain.

The current study concluded that TiO_2 and ZnO NPs nanoparticles do not show a toxic or negative effect on any of the studied variables. However, it is known that a lot of nanoparticles have an adverse effect on plant development and photosynthesis. The toxicity of nanoparticles is also believed to depend on the composition of their chemicals, type, dose, stability, culture medium composition composition, species of plants, and technique of application (68).

5.1 Effect of TiO_2 and ZnO NPs on seeds germination and morphology:

As there was no difference between the control plants and the plants on which the nanoparticles were applied in MS medium in terms of germination rate and morphology (the roots and hypocotyls). The experimental result from the present study shows that TiO_2 and ZnO NPs have no negative impact on the germination of seeds, shoot development, or root growth. This may be because the plant is not sensitive to these chemicals (81).

Since the seeds are the primary point of interaction between the system of plants and the nanoparticles. The emergence of roots signifies the start of seed germination and seedling development. The main data set utilized to evaluate the impact of different nanomaterials on the later developmental stages of plants is the germination of seeds rate (21). Many studies have been conducted on this topic, positive as well as negative.

ZnO NPs at low concentrations have been proven to enhance seed germination in a range of plant species, including peanut (Arachis hypogea), and

they explain this based on the fact that it is known that the high zinc content in seeds can act as fertilizer, the treatment of peanut seeds with zinc oxide nanoparticles at a concentration of 1000 parts per million resulted in a considerable improvement in germination, shoot length, and root length. Although the precise cause of these effects is unknown, it is possible that they were caused by higher zinc concentrations in the seeds after treatment with zinc oxide nanoparticles; they are also due to the low bioavailability of the nanoparticles due to their size and low water solubility, in addition to the fact that the inherent large surface area and small size associated with ZnO may lead to increased adsorption (82).

In another study (83), ZnO NPs showed an effect of increasing germination in cucumber (*Cucumis sativus*) by 10% and decreasing germination of seeds of alfalfa (*Medicago sativa*) and tomato (*Solanum lycopersicum*) by forty and twenty percent, respectively, but more importantly, they discussed the differential effects of the same plants under the influence of The zinc oxide nanoparticles results varied according to the factors of the experiment; they suggested that seed germination rates may vary greatly based on exposure time, concentrations used in the medium, and the studied plant species.

Also, the size of nanoparticles and their solubility have an important role in causing differential effects on the germination of seeds, which could be related to differences in cultivar composition, and they suggested that analytical and biochemical assessments might provide a better understanding of the nature of plant tolerance and resistance to zinc oxide nanoparticles. Whereas in the case of *Cucurbita pepo* (zucchini) seeds in hydroponics, the germination and root growth with zinc oxide nanoparticles revealed no adverse effects (84).

However, the effects of TiO2 NPs and ZnO NPs on spinach seeds demonstrated a clear effectiveness in increasing the rate of germination of seeds, and the results of titanium were better than those of ZnO NPs (85), and this may be explained by the fact that the generation of reactive anions accumulates extra water and enhances the level of oxygen through germination; moreover, because of the small dimensions of TiO NPs, they are able to enter the spinach seed cap and

enhance action during development by increasing the consumption of inorganic nutrients and accelerating the breakdown of organic matter (86).
It was also demonstrated that high doses of TiO_2 NPs (up to 200 ppm) in tomatoes and onions were able to increase root and shoot lengths. Treatment with TiO2 NPs increases seedling growth, which may be explained by the fact that it controls the activity of an enzyme involved in the metabolism of nitrogen, which aids in the conversion of nitrogen for chlorophyll synthesis (87).

Eventually, the non-negative effect of nanoparticles in this research has two meanings: first, that the ZnO NPs and TiO_2 NPs used are non-toxic to the studied plants, which means that sugar beets were not sensitive to either of them; Second, as reported in several previous studies, variability in plant response to nanoparticles depends on many factors, including concentration, size, experimental conditions, and duration of exposure to nanoparticles.

5.2 Effect of TiO_2 and ZnO NPs on total chlorophyll and caroteniod:

As for the photosynthesis process represented by chlorophyll and carotenoids, these pigments were unaffected by the treatment of TiO_2 NPs and ZnO NPs to them at any concentration for both genotypes.
Therefore, it can be considered that the effect is generally not negative on photosynthesis and that the photosynthesis process did not differ from the control plants.

TiO_2 NPs is known for its role in promoting photosynthesis, protecting chloroplasts from aging, as well as promoting chlorophyll formation and improving RuBisCo activity (42).
But in a study on the effect of TiO_2 NPs on chlorophyll in the lettuce plant, it did not have any significant effect on it in this regard (88).
Interestingly, another study showed the impacts of titanium dioxide nanoparticles in basil and its response in terms of photosynthesis, and they found a significant decrease in relative chlorophyll content at two concentrations, 250 mg/kg (24%) and 750 mg/kg (18%) (89).

In another study on spinach, it was reported that ZnO and TiO2 applications resulted in a gradual increase in the quantity of chlorophyll of 30.17% and 40.69%, respectively, when compared with the control group (85).

One of the most important studies that shows the difference in response between plants under the influence of the same type of nanoparticles was a comparison study between nine different plants and the impact of zinc oxide nanoparticles on both a and b hlorophyll in each of them.

The results varied between the studied plants, and their explanation was that although numerous studies have demonstrated that ZnO NPs may provoke oxidative stress and modulate the activities of antioxidant enzymes, or chloroplast function, in their study, ZnO NPs changed several variables associated with oxidative stress in the majority of the studied plant species, but the changes were greatly reliant on the species of the plant being studied (90).

On the other hand, one of the researches focused on the influence of ZnO NPs and TiO_2 NPs on the tomato plant *(Solanum lycopersicum L.),* and as a consequence, nanoparticles were shown to have higher chlorophyll contents than control plants (91).

However, this result contrasted with another study applied to the same plant; they used Ag NPs and TiO_2 NPs, and the result found that Ag NPs caused a decrease in the quantity of chlorophyll, whereas TiO2 NPs had no effect on the chlorophyll content when compared to control plants (92).

These discrepancies were explained by the varied features of nanoparticles across various dosages, application patterns, and exposure concentrations (91).

In other words, the same plant does not show the same response to all types of nanoparticles, even if they are of the same type (metal or metal oxide), for each of them is expected to have a different impact on the same plant in a unique way. based on the plant's characteristics and susceptibility to the metal ions that make up the nanoparticles.

It is worth referring to a related study on the impact of zinc oxide and titanium dioxide nanoparticles on wheat plants (*Triticum aestivum)* that shows that these chemicals have no adverse effects on seed germination or the development of roots and shoots. At the same time, ZnO NPs only contributed to the increase in chlorophyll content. The reason for this, as they explained, can be attributed to the fact that TiO_2 and ZnO nanostructures are intractable in water and the quick loss of the particles from solution, either as a consequence of sedimentation as a result of aggregation or the sensitivity of the test plant (81).

In a previous study, zinc oxide nanoparticles had a positive impact on wheat plants (*Triticum aestivum*) by reducing oxidative stress and increasing levels of chlorophyll and carotenoids (39).
Similar results have been shown in the rice plant (*Oryza sativa*), where TiO2 NPs have been shown to decrease oxidative stress while boosting chlorophyll and carotenoid content (93).

Despite the fact that the effect of zinc oxide nanoparticles on *Arabidopsis* showed a negative effect in terms of the plant's content of chlorophyll, it demonstrated a slight increase in the content of carotenoids; this was explained by the fact that the zinc oxide nanoparticles treatment affected the expression of genes involved in carotenoid synthesis, as the toxic effect of ZnO NPs on *Arabidopsis* plants was linked to the induction of oxidative stress in addition to the detrimental effect on chlorophyll synthesis and the expression of genes in the photosynthetic system (37).

As a result, the paths that are related to the impacts of NPs in plants are different and overlap, and this is consistent with the findings in this research in terms of the difference in results between all parameters.

5.3 Effect of TiO_2 and ZnO NPs on secondary metabolites:

There haven't been many studies on the effect of NPs on betaine or betalain, but there have been some on other secondary metabolites.

One study discovered that ZnO NPs had an advantageous effect on in vitro steviol glycoside synthesis in *Stevia rebaudiana*; utilizing 1 mg/l doses of zinc oxide nanoparticles, the amount of steviol glycosides produced was approximately doubled when compared to the control (94).
They interpreted their results as follows: the secondary metabolite increased to its maximum level at the optimal ZnO NPs concentration as a result of the plant's exposure to abiotic stress caused by ZnO NPs, which increased antioxidant activity. While at the starting level, the growth regulators in plant cells were unbalanced as a result of the action of free radicals and reactive oxygen species.

In similar research on the impact of zinc oxide NPs on the secondary metabolite steviol glycoside, they explain that the mechanisms that underlie the increase in steviol glycoside, phytochemical antioxidant activities, and thus the high levels of reactive oxygen species (ROS) and Zn+1 free radicals may contribute to an instant decline in growth and metabolism after passing a particular threshold (95).

And many studies showed the efficiency of ZnO NPs by increasing secondary metabolites in the Carla plant (*Momordica charantia L*), including phenols, flavonoids, carotenoids, proline, and carbohydrates (96).
As well, it has been reported to boost the mulberry plant's (*Zataria multiflora*) thymol and carvacrol levels (97).

Likewise, the addition of titanium dioxide nanoparticles improved the quantity of chlorogenic acid, cinnamic acid, gallic acid, coumaric acid, and tannic acid in Cicer arietinum callus cultures. (98).
Moreover, another study demonstrated that the effect of TiO_2 nanoparticles in *Vetiveria zizanioides L*. resulted in a significant improvement by producing more essential oil than control plants (99).

As well, the same positive effect of TiO_2 NPs was observed in sage plant (*Salvia officinalis*), which significantly stimulated essential oils in addition to phenols and flavonoids, it was observed that the main constituents of essential oils of *Salvia officinalis*, such as cis-Thujene (34.5%) and 1,8 Cineol (21.2%) were

found in the highest concentrations in plants treated with 200 mg/L of titanium dioxide nanoparticles, whereas the highest concentration of camphene (12.1%) was found in plants that were exposed to 1000 mg/L (100).

While the antioxidant and amino acid content of the rice plant (*Oryza sativa L.*) improved as a result of exposure to TiO2 NPs, the plant's carbohydrate content was negatively impacted. (101).

All these secondary metabolites produced by plants have commercial or health benefits for humans, and it is clear that many NPs have an improvement or stimulation role for their production, and therefore a role in crop improvement and production. It can be considered a topic that deserves further study and research because it seems that each type of nanoparticle with different concentrations of any of them has effects that vary differently on the same plant, so each concentration can be effective on one type of secondary metabolite but not the other.
Furthermore, these studies showed that the use of NPs was not only beneficial in increasing the production of the bioactive compound, but also helped plants that were exposed to adverse stress conditions (68).

Nanoparticles have the unique capacity to affect gene expression and the production pathways of secondary metabolites. In a related study, TiO2 NPs were shown to be effective in increasing the expression of geranyl diphosphate synthase (GPPS), a gene important in the formation of the secondary metabolite thymoquinone in black cumin (*Nigella sativa L.).* This resulted from enhanced regulation of associated metabolic pathway genes. Additionally, the production of secondary metabolites is significantly influenced by genotype and nanoparticle concentration (102).
And this finding explains the results of this research, which differed between genotypes SG2020 and SG833 of the *Beta vulgaris* plants.

Taken together, the precise mechanism by which nanoparticles accelerate secondary metabolism is unknown. One possible mechanism is that reactive oxygen species (ROS), which are generated by nanoparticles, allow secondary metabolism to be activated in response to the stress that they create. Increasing the expression

of several genes involved in secondary metabolite biosynthesis is another strategy for boosting their production (60) (66).

The significance of the nanoparticle impact is that depending on the chemical profile, there might be considerable differences in the effect or accumulation, and this varies between plant yields (103) (104).

Therefore, there is a need for more comparative studies between plant varieties and species. It appears that nanoparticles have varied impacts on different plant species and genotypes, and the most important thing is that the concentration applied to the plant has the greatest effect on the final result.

It is noteworthy that nanoparticles can trigger and promote secondary metabolite synthesis, but only in a manner that depends on the dose. This necessitates numerous studies to determine the (ideal) or effective concentrations for maximum metabolite production, as well as the (toxic) concentrations of plants.

6 CONCLUSIONS AND RECOMMENDATIONS

In this study, the effect of titanium dioxide nanoparticles and zinc oxide nanoparticles on two genotypes (SG2020 and SG833) of *Beta vulgaris* plants was investigated in vitro. The results for secondary metabolites showed an increase and a clear improvement in the production of betaine and betalain in some applied treatments, while seed germination and morphology, as well as chlorophyll content and carotenoids, were not affected by NPs, and the results were not significant.

Furthermore, the effects of TiO_2 and ZnO NPs treatments differed between plants of the two genotypes, with betanin increasing only in genotype SG338 and at 25 mg/m، of zinc oxide nanoparticles.

Even though plants exposed to 15 and 25 mg/mL of titanium dioxide nanoparticles significantly increased the vulgaxanthin I content in the genotype SG833 compared to the control, plants treated with 15 and 25 mg/mL of zinc oxide nanoparticles significantly increased the vulgaxanthin I content in the genotype SG2020.

The results were consistent with regard to betaine in the two genotypes, as it was found that the lower concentration of nanoparticles showed statistically higher results in the content of betaine with respect to the control group. It reached 283% in genotype SG2020 in plants exposed to 5 mg/mL of TiO_2 nanoparticles and 232% at concentrations of 5 mg/mL of ZnO nanoparticles compared to the control plants. Then it gradually decreases at higher concentrations, but all treatments are considered to have increased the content of betaine in the plants that were exposed to them.

Similarly, for genotype SG833, the content of betaine was statistically significant and reached 33.27% in plants exposed to 5 mg/mL of TiO2 nanoparticles in comparison to the control group and 29.88% in plants exposed to 5 mg/mL of ZnO nanoparticles with respect to the control group.

All these results have a real meaning that shows the difference in the effect and response of the plant to each treatment that may be applied to it, and each factor here has a different effect and another result that can be obtained, which opens the

way for opportunities that can be exploited to increase the desired contents of the plant and the possibility of NPs in contributing to the enhancement of secondary metabolites and thus improving the quality of agricultural crops and increasing their tolerance to the stresses surrounding the plant. Considering that betaine and betalain are important and useful secondary metabolites, this study showed the possibility of increasing them and the most important thing without having toxic effects from NPs on the plant.

Certainly, the effects can be further afield, and it is interesting to research more deeply in this field to understand the mechanisms of influence more and more. Considering the toxic effects of some NPs on certain plants, it is crucial to study and better understand them in order to protect plants from their effects.

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