

T.R.
BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
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**COMPARISON OF DIFFERENT SEED PRIMING
TECHNOLOGIES FOR SEED GERMINATION AND PLANT
GROWTH IN SUGAR BEET (*BETA VULGARIS* L.)**

DOCTOR OF PHILOSOPHY

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ABSTRACT

COMPARISON OF DIFFERENT SEED PRIMING TECHNOLOGIES FOR SEED GERMINATION AND PLANT GROWTH IN SUGAR BEET (*BETA VULGARIS* L.)

PHD THESIS

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(xiv + 78)

Environmental stress is a significant threat to crop production and quality. The effect of ecological changes, shortage of natural resources, more demand for food, and increased crop production, more attention has turned to improving seed quality and crop plant production through implementing efficient strategies. Seed priming, an innovative technology, has emerged as a pivotal strategy in this pursuit. This research was carried out to determine the effects of hydro-priming and nano-priming on seed germination, α -amylase activity, chlorophyll and MDA content in the sugar beet genotype SG188 (developed by Songül GÜREL). Seeds were soaked in water and in solutions containing different concentrations (5,10, and 15 ppm) of TiO₂ NPs for 3,6,9 hours and experiments were repeated three times. As the first step, the seed was primed and stored at 4°C for 10 and 45 days as a post-priming application. Before sowing seeds, after 10 days of priming, α -amylase activity, chlorophyll and MDA content of seeds were measured in primed seeds and the control. Then, growth and development parameters were checked after 10 days of germination. It showed that the germination rate in the primed-seed group was relatively higher than in the control. Next, Image J software showed that seeds soaked with nanoparticles 5,10,15 ppm for 3 hours had a positive impact and increased root length compared to the control. In the next step, after 45 days of priming, seedlings were analyzed similarly to seedlings after 10 days post-priming in terms of morphology, physiology, and biochemistry by ANOVA. In addition, a comparison of post-priming treatments showed that 45 days post-priming led to a boost of α -amylase enzyme activity compared to 10 days post-priming.

KEYWORDS: Sugar beet, Nanoparticles, Hydro-priming, Nano-priming, Titanium dioxide, Germination

ÖZET

**ŞEKER PANCARINDA (BETA VULGARIS L) TOHUM ÇİMLENMESİ
VE BİTKİ BÜYÜMESİ İÇİN FARKLI TOHUM ÖN-UYGULAMA
TEKNOLOJİLERİNİN KARŞILAŞTIRILMASI
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BİYOLOJİ ANABİLİM DALI**

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Çevresel stres bitki üretimi ve kalitesi için önemli bir tehdittir. Ekolojik değişimler, doğal kaynakların azalması, gıda talebinin artması ve bitkisel üretimindeki iyileştirmeler için etkili stratejiler uygulayarak tohum kalitesinin ve bitkisel üretimin artırılmasına daha fazla önem verilmiştir. Yenilikçi bir teknoloji olan tohum ön uygulaması (priming) bu konuda çok önemli bir strateji olarak ortaya çıkmıştır. Bu araştırma, şeker pancarı genotipi (Songül GÜREL tarafından geliştirilen) SG188 'de hidro-priming ve nano-priming'in tohum çimlenmesi, α -amilaz aktivitesi, klorofil ve MDA içeriği üzerine etkilerini belirlemek amacıyla yapılmıştır. Tohumlar suda ve farklı konsantrasyonlarda (5,10 ve 15 ppm) TiO_2 NP içeren çözeltilerde 3, 6, 9 saat süreyle bekletilmiş ve deneyler üç kez tekrarlanmıştır. İlk aşamada tohumlara priming uygulaması yapılmış ve priming sonrasında 4°C 'de 10 ve 45 gün süreyle saklanmıştır. Priming sonrası 10 gün süreyle saklanan tohumlarda çimlenme öncesinde α -amilaz enzim aktivitesi belirlenmiştir. Daha sonra tohumlar çimlendirilmiş ve çimlenmeden 10 gün sonra büyüme ve gelişme parametreleri kontrol edilmiştir. Priming tohum grubunda çimlenme oranının kontrol grubuna göre daha yüksek olduğu belirlenmiştir. Çimlenen bitkilerde Image J yazılımı ile kök uzunluğu belirlenmiş ve kontrole göre 3 saat boyunca 5, 10, 15 ppm nanopartiküllerle priming uygulanan tohumlarda kök uzunluğunun arttığı gösterilmiştir. Priming sonrası 45 gün saklanan tohumlar çimlendirilerek elde edilen bitkilerde morfolojik, fizyolojik ve biyokimyasal özellikler bakımından priming sonrası 10 gün saklanan fidelerde olduğu gibi ANOVA analizi yapılmıştır. Ek olarak, hazırlama sonrası uygulamaların karşılaştırması yapıldığında 45 gün post-priming uygulamasında 10 gün uygulamaya göre α -amilaz enzim aktivitesinde bir artışa yol açtığı gösterilmiştir.

ANAHTAR KELİMELELER: Şeker pancarı, Nanopartikül, Hidro-priming, Nano-priming, Titanyum dioksit, Çimlenme

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LIST OF ABBREVIATIONS AND SYMBOLS

UV	: Ultraviolet
µg	: Microgram
ANOVA	: Analysis of variance
NPs	: Nanoparticle
TiO₂ NPs	: Titanium dioxide nanoparticle
CO₂	: Carbon dioxide
g	: grams
hrs	: Hours
L	: Liter
U	: Enzyme unit
v/v	: Volume to volume ratio
mL	: Milliliter
mM	: Milimolar
ROS	: Reactive Oxygen Species
rpm	: Rotations per minute
w/v	: Weight to volume ratio
POD	: Peroxidase
SOD	: Superoxidase
CAT	: Catalase
TBARS	: Thiobarbituric acid reactive substances
H₂O₂	: Hydrogen peroxide
TEM	: Transmission electron microscopy
µL	: Microliter
SEM	: Scanning electron microscopy
MDA	: Malondialdehyde
XRD	: X-ray powder diffraction
FTIR	: Fourier-transform infrared spectroscopy
DLS	: Dynamic light scattering
°C	: Degree celsius
PBS	: Phosphate- buffered saline
CH₃COOH/CH₃COONa	: Acetic acid/sodium acetate buffer
CaCl₂	: Calcium chloride

KI	: Potassium iodide
I₂	: Iodine
DMF	: Dimethylformamide
TBA	: Thiobarbituric acid
TCA	: Trichloroacetic acid



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1. INTRODUCTION

1.1 Significance of Sugar Beet

Sugar beet is an important production among different plants such as swiss chard, spinach beet, fodder beet, and garden beet, all of which belong to *Beta vulgaris* ssp. *vulgaris* from the family Chenopodiaceae (Gill et al., 1980). The product originated from fodder beets with high sugar content in the late 18th century, and the first commercial sugar extraction operations began in 1802. The extraction of sugar from beets was a significant advance in agriculture in the 19th century in northern Europe. Pioneering research in Germany paved the way for the beet sugar industry. The Napoleonic Wars significantly popularized it as a practical replacement for cane sugar, especially within the borders of France. As a result of subsequent technological advances and supportive government policies, the beet sugar industry has grown considerably. Sugar beets are currently cultivated and processed throughout North and South America, Europe, Asia, and Africa (Draycott, 2008).

Sugar plays an essential role in various foods and beverages due to its capacity to sweeten, provide energy, and act as a preservative. Despite concerns about potential health consequences and competition from other sugar sources such as isoglucose from cereals and fructose from corn, as well as artificial sweeteners such as saccharin, aspartame, and cyclamate demand for sugar continues to increase. Global sugar consumption from cane and beet sources has increased fivefold in sixty years with no signs of slowing down. (Draycott, 2008).

Sugar beet holds a prominent position in Türkiye's industrial and economic sectors, standing as the second most crucial agricultural product following wheat. (FAOSTAT, 2016). Türkiye is the world's fifth-largest sugar beet producer, after France, Germany, Poland, the United States, and Russia. Sugar is of great economic importance in Turkey and contributes greatly to the country's financial prospects (Akçay & Uzunoğlu, 2006). *Beta vulgaris* L. primarily grows in the central Anatolian area, which is characterized by low rainfall levels. As a result, agricultural products in this area face the challenges of drought-induced pressure and frequently grapples with salinity stress (Sensoy et al., 2008).

Considering the importance of this plant in production, yield, resilience, and tolerance against biological and environmental stresses in Türkiye, it was chosen as

an essential product for research. The aim is to provide valuable information from the research findings.

1.2 Sugar Beet Overview

In higher plants, the seeds play a vital role because they contain an embryo surrounded by protective membranes. This is crucial for the successful establishment of a new plant generation (Bewley, 1997; Kucera et al., 2005). The diverse structural composition of these protective layers is not only a distinctive feature in the evolution of dispersal units (Finch-Savage & Leubner-Metzger, 2006), but has great significance in how germination responds to environmental signals and plant hormones. The germination process begins with the absorption of water through the dry seed or fruit, which leads to the expansion of the embryo. As the embryo elongates and begins to break via the surrounding layers, more water is absorbed. Germination happens when a part of the embryo, typically the radicle, successfully penetrates whole the protective layers. It is an important to note that the seed coat, known as the testa, is consistently present, and the endosperm, serving as an extensive protective layer, covers the mature seeds (Hermann et al., 2007).

In botanical terms, the seed is more accurately described as a fruit. It consists of the true seed, covered by the fruit coat or pericarp, which originates from the carpels of the flower and incorporates other parts of the flower as well. (Artschwager, 1927; Deleuran et al., 2013; Hermann et al., 2007; Lukaszewska & Sliwinska, 2007). The upper section of the pericarp is formed by the operculum, which acts as the cap of the ovary. As the operculum rises, it exposes the opening that displays the radicle end of the seed. When the radicle protrudes through all the layers that cover it, namely the pericarp (through the opening of the operculum), the testa (through rupture), and the endosperm (through rupture), it becomes visible. This emergence of the radicle is known as the observable protrusion of the radicle tip (Hermann et al., 2007) (Fig. 1.1).

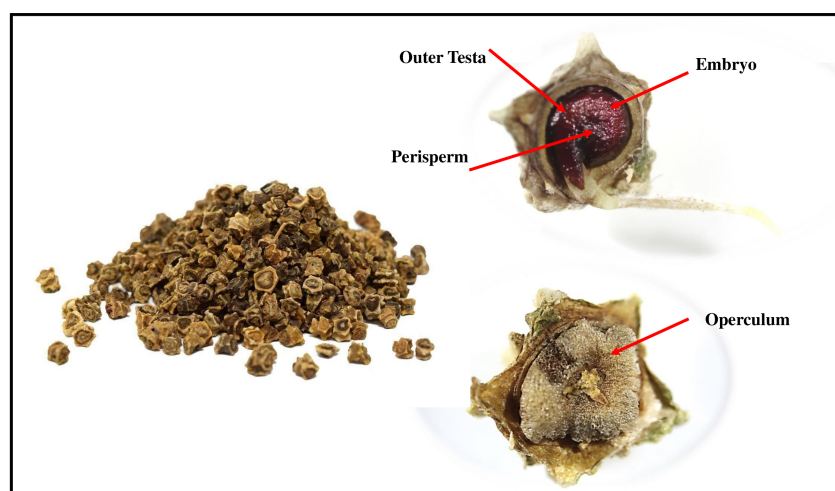


Figure 1.1. Structure of mature seed of *Beta vulgaris* L. plant

Sugar derived from sugar beet or sugar cane is commonly known as sucrose-based or white sugar. About 79% of sucrose-based sugar production worldwide comes from sugar cane, while the remaining 21% is obtained from sugar beet. Leading producers of sugar from sugarcane include Brazil, India, and Thailand. On the other hand, the primary contributors to sugar production from sugar beet are the European Union (EU), Russia, the United States (USA), Türkiye, and Ukraine. Sugar beet, scientifically classified within the Chenopodiaceae family, has significant global importance as an origin of sugar production which is prominent in temperate regions. It contributes substantially, providing approximately 30% of the world's annual sugar output (Blunk et al., 2018; Dohm et al., 2014; Frese, 2010). Sugar beet is a biennial plant that is used to produce crops after a two-year growth cycle. During the first year, it focuses on vegetative growth and root formation. In the second year, it undergoes vernalization and genetic maturity, which leads to flowering and seed production. Most sugar beet cultivars go through a blooming phase that lasts 3 to 4 weeks. In general, larger seedlings start flowering earlier than their smaller counterparts. The most favorable conditions for flowering typically appear in the average temperature range of 15-20°C, with an upper limit of 35°C. When beet flowers are pollinated, they are produced in structures called glomerules. Polygerm seeds originate from glomeruli containing multiple embryos, while monogerm seeds derive from glomeruli with a single embryo. Sugar beet seeds are characterized by their hard outer shells, which typically exhibit a brown coloration (Tuğrul, 2022). These seeds are utilized by farmers who supply raw materials to

sugar factories. The root dry matter content typically ranges from 20-26%, while the leaves contain approximately 11-19% dry matter, with variations based on factors like climate, growing conditions, and beet variety (Brar et al., 2015).

Different factors come into play in determining sugar beet yield and quality, including crop rotation, beet variety, fertilization, tillage, sowing techniques, weeding, irrigation, disease, and pest management, harvesting, and silage. Effective control of these factors through proper planning can result in high-yield, high-quality crops. On the other hand, effective management practices are necessary for farmers to mitigate the impact of uncontrollable factors like climate and soil on yield and quality (Tuğrul, 2022).

Seed quality is crucial for the success of farmers and the agricultural sector as it plays a significant role in ensuring food security. Maintaining seed quality requires attention at every level of the production process, from selecting suitable farms to harvesting, processing, and storage (Tahir et al., 2023). Notably, despite efforts to ensure seed quality, production or storage conditions can still compromise it (Afzal et al., 2020). Seed quality includes four key aspects: genetic, physical, physiological, and pathological quality. The genetic quality of seeds refers to the hereditary traits that are inherited across generations. Physical quality refers to the optimal seed size, purity, appearance, color, moisture levels, and the absence of pest damage. The physiological quality of a seed determines its ability to germinate, indicating its viability and potential for growth. Pathological quality encompasses features relevant to the presence or absence of diseases that may prevent the healthy growth of the plant (Raj & Raj, 2019). It is crucial to note that the success of crop production is directly related to the quality of seeds used in the field. Therefore, it is important to consider these factors to improve seed quality.

To enhance seed performance and address these challenges, agricultural research has concentrated on the development of technologies that facilitate early emergence, consistent growth and development, resistance to various stresses, uniform maturity, and productivity, as evident in studies by Kah et al., (2019) and Lowry et al., (2019). El Boukhari et al., (2021) demonstrated techniques to improve seed performance in the field and during storage.

Seed priming is a technique that involves a controlled process of hydrating and drying the seed, which activates the metabolic processes within it without inducing radicle protrusion. This method has several advantages, including

reducing variability in seed germination rates, leading to more consistent and speedy germination, and improving stand establishment in the field (Taylor et al., 1998). Moreover, research has demonstrated that seed priming can improve seed resilience against adverse conditions, such as thermal, moisture, and osmotic stress during sowing (Afzal et al., 2012, 2017; Chakraborty & Dwivedi, 2021). For instance, two distinct varieties of sugar beet seeds were subjected to pretreatment experiments using polyethylene glycol solution at various concentrations and priming durations as traditional priming methods by Jalilian & Tavakol, (2005). Their findings indicated that treating sugar beet seeds with PEG 8000 at a concentration of 0.5 MPa during five days led to an 8% growth rate enhancement. Furthermore, seed germination time was effectively reduced by 50% with priming technique. Another experiment involving tomato seed priming as an advanced one, the utilization of nanocarrier systems based on chitosan with gibberellic acid was observed to enhance not just the initial growth of seedlings but also enhance fruit yield in field conditions. In the present study, it was determined that seed nano-priming led to a substantial increase in production, with growth rates of 225.5% and 178.8% for chitosan/tripolyphosphate and alginate/chitosan NPs loaded with gibberellic acid. Notably, the techniques employed in the production of these nanoparticles do not require the use of organic solvents, making them an environmentally sustainable choice for agricultural purposes (Pereira et al., 2019).

In the seed industry, the choice of priming method is usually based on specific challenges. The main goal is to improve germination speed and uniformity. Meanwhile, sugar beet farmers need seeds that have exceptional germination capacity and vigor, ensuring prompt and robust field greening. In the production of sugar beet seed, all efforts are focused on achieving high seed quality and seed purity (Kockelmann et al., 2010).

Currently, there is a growing interest in seed enhancement technologies. This is due to extensive research and development aimed at adapting these technologies for various types of seeds. While there are initial equipment costs and scalability challenges exist, these technologies have attracted attention within the crop and horticulture seed supply chains due to their significant advantages. Ongoing developments show promising potential for further improvements if optimization and effective scaling can be achieved. However, continuous

refinement is essential for the practically applying seed priming in industry and agriculture.

1.3 Role of Nanotechnology in Plant Agriculture

As a rapidly growing interdisciplinary field, nanotechnology is bringing engineered nanoparticles into the worldwide market. This innovation promises to improve the fields of agricultural science and related areas (Usman et al., 2020; Vithanage et al., 2023).

Agriculture has started using nanotechnology to improve sustainability and productivity by incorporating engineered nanoparticles and various nanomaterials such as nanocapsules, nanocarriers, nano-fertilizers, pesticides, and nanosensors (Usman et al., 2020; Dziergowska & Michalak, 2022). Nanoparticles have a size range of one to one hundred nanometers. (Corsi et al., 2023). Nanomaterials have exceptional properties that are primarily attributed to their high surface area relative to their volume (Haris et al., 2023). Catalytic efficiency experiences a significant increase through the quantum nanoscale. The tiny size, shape, and increased surface area of NPs make them highly effective in targeting specific cellular sites and delivering advantageous substances (Dziergowska & Michalak, 2022; Usman et al., 2020).

In recent years, diverse rare earth metal-based nanoparticles, containing ZnO-NPs, Zn-NPs, Ag-NPs, FeS₂-NPs, Fe-NPs, CeO₂-NPs, SiO₂-NPs, Au-NPs, TiO₂-NPs, CuO-NPs, and carbon-based NPs like carbon nanotubes and fullerenes, have been utilized under both controlled and natural environments. These applications aim to enable plants to thrive in different challenging habitats (Zulfiqar & Ashraf, 2021; Usman et al., 2020).

In general, nanotechnology has become known as a viable way to overcome the limits of conventional agriculture practices. In every aspect of agriculture, including seed preservation, priming technique, germination of seed, fertilization, and post-harvest processes, nanotechnology holds the potential to increase both crop yield and quality (Shukla et al., 2019) and also include new approaches for the remediation of soil and water along with the improvement of nano-fertilizers and nano-pesticides can contribute to pest control. Moreover, the utilize of nano-enhanced products in crop protection can significantly decrease the environmental consequences associated with agriculture and provide great environmental compatibility (De La Torre-Roche et al., 2020). Nanomaterials, particularly

nanoparticles, offer a broad range of applications in the field of crop protection (Kah et al., 2019). In fact, this integration of nanotechnology in agriculture serves four primary purposes: promoting growth of plant, improving crop yields, enhancing quality of soil, and implementing intelligent observing techniques (Dziergowska & Michalak, 2022). Recent research has demonstrated that nanoparticles exert influences on seeds and plants (Mahakham et al., 2017; Lowry et al., 2019; Acharya et al., 2020). Some nanoparticles exhibit adverse impacts, including phytotoxicity or inhibiting the germination of seedlings (Pelegrino et al., 2020; Falco et al., 2020). Conversely, certain nanoparticles can function as stimulants, enhancing seed metabolism, seedling vigor, and plant growth by modulating cellular signaling pathways (Abbasi Khalaki et al., 2021). These effects depend on the physiochemical characteristics of the nanoparticles, including dimension, zeta potential, and concentration, which are crucial in controlling the biological responses (Acharya et al., 2019). These characteristics are critical to the uptake and transport of nanoparticles within plants. For instance, smaller nanoparticles cross biological barriers more effectively (Hu et al., 2020). NPs surface charge has a significant impact on their interaction with plants. Positively and negatively charged nanoparticles behave differently. Positively charged nanoparticles can be uptake by leaves and transported to the roots, while negatively charged nanoparticles are only absorbed by the roots. Additionally, positively charged nanoparticles stimulate the production of mucilage, which can prevent their uptake by plants (Spielman-Sun et al., 2019).

1.3.1 Mechanism of Nanoparticles

Nanoparticles can access plant cells through multiple mechanisms. The initial mechanism involves the nanoparticles capable of directly penetrating the plasma membrane via diffusion, with factors such as particle size, hydrophobicity, composition, charge, and shape affecting this phenomenon (Auría-Soro et al., 2019). The second mechanism is endocytosis, in which cells actively engulf nanoparticles by encircling them with the cell membrane (Foroozandeh & Aziz, 2018). Another strategy includes transmembrane proteins or channels that regulate nanoparticle transport into cells, although specific factors like charge and pore size will affect this function (Li et al., 2020).

The transport of nanoparticles within plants occurs via foliar/shoots or roots, utilizing either an apoplastic or symplastic transport path (Pérez-de-Luque, 2017).

Apoplastic transport involves movement through the xylem vessels, extracellular matrix, and cell walls, enabling radial movement to reach the vascular tissue and then ascend to the above-ground parts of the plant (Sun et al., 2014). In contrast, plasmodesmata and sieve plates facilitate symplastic transfer between adjacent cells. Nanoparticles (NPs) are primarily absorbed and stored within plants through leaves, roots, and root hairs, using various pathways such as pit membranes, hydathodes, cell wall junctions, casparian strips, and extracellular spaces (Ruttkey-Nedecky et al., 2017). A secondary system for NPs entry into plants involves pathways like lateral roots, root hairs, root tips, and rhizoderms, (A. Yan & Chen, 2019). High ionic strength can cause NPs aggregation, while organic macromolecules and low-molecular-weight organic acids in root exudates affect NPs stability, solubility, and uptake by the root-associated microorganisms or plant. Plant sections exhibit selective uptake, biotransformation, regulation, distribution, and translocation of various nanoparticle types (McLean et al., 2013; Michaud et al., 2007).

1.3.2 Active Nanoparticles

Active nanoparticles are a type of nanoparticles that possess inherent biological activity, functioning as either stimulants, anti-pathogens, or a mix of the two (Rizwan et al., 2019; Siddaiah et al., 2018). On the other hand, nanocarriers equipped with sustained release functionalities encompass nanoparticles, regardless of their activity, that are loaded with an active ingredient, whether natural or artificial, to deliver a slow discharge of the substance over a prolonged duration (Falsini et al., 2019; Shakiba et al., 2020). Active nanoparticles include metallic nanoparticles, which are typically less than 100 nm. They have gained significant attention for their application in seed nano-priming, particularly when they incorporate metals that play vital roles in plant metabolism and biofortification (De La Torre-Roche et al., 2020). These essential metals, including zinc, iron, and manganese, serve as co-factors for various enzymes that are integral to important metabolic activities, such as respiration, antioxidant responses, and photosynthesis (Kasote et al., 2019).

However, it is important to recognize that some metallic nanoparticles have the potential to be harmful to seeds or plants because of re-oxidation phenomena. For instance, silver nanoparticles may undergo re-oxidation within seeds and plants,

transforming from Ag^0 to Ag^+ ions, which can deactivate proteins and enzymes, causing harmful effects. However, when bioactive chemicals are present as coatings on biogenic nanoparticles, they serve as stabilizing agents, preventing the re-oxidation reaction, and thereby improving the biocompatibility of metallic nanoparticles (Kannaujia et al., 2019). Various biogenic metallic nanoparticles have been found to boost seed germination, promote plant growth and development, and raise content of chlorophyll and antioxidants levels (Acharya et al., 2020; C. K. Das et al., 2018; Kasote et al., 2019; Mahakham et al., 2016).

1.3.3 Nano Carrier System

Biopolymeric nanoparticles are a system that can be generated using polysaccharides, lipids, and proteins. These nanoparticles offer biodegradability, biocompatibility, and the versatility to be tailored for specific responses to environmental cues (Camara et al., 2019). They can contain various compounds, including fungicides, essential oils, plant growth regulators, and fertilizers (Gao et al., 2020; Kavetsou et al., 2019; Maluin et al., 2020). Polymeric nanoparticles, when utilized as seed coatings, generally have a size exceeding 100 nm, facilitating the gradual release of active ingredients. These substances can effectively alter plant metabolism or combat pathogens (Falsini et al., 2019).

A variety of nanocarrier systems based on biopolymers are applicable for seed treatment. These systems encompass materials such as synthetic biopolymers, alginate, zein, cellulose, like poly-epsilon-caprolactone, polylactic-co-glycolic acid, and polylactic acid, as well as lipid nanoparticles (Campos et al., 2015; Coelho et al., 2018; da Silva Santos et al., 2019; Du et al., 2019; Ji et al., 2019; Qi et al., 2020; H. Zhang et al., 2017). Biopolymeric nanoparticles can function as carriers for essential metal micronutrients, ensuring their controlled release. For instance, research by (Saharan et al., 2016) demonstrated that maize primed-seeds with chitosan nanoparticles which includes copper resulted in increased alpha-amylase and protease activity, promoting reserve mobilization, and facilitating seedling growth. In contrast, bulk copper treatment exhibited inhibitory effects. Similarly, priming with zinc-loaded chitosan nanoparticles enhanced the activity of hydrolytic enzymes in seeds of maize, which accelerated germination.

Nanocarrier systems designed for plant growth regulators have shown promise in seed priming, delivering benefits from improved germination to

enhanced production. For instance, (Falsini et al., 2019) coated arugula and seeds of tomato with lignin nanoparticles including gibberellic acid, resulting in variable effects depending on the sowing time post-treatment. In arugula seeds, these formulations enhance germination, lengths of stem and root, and overall plant biomass. Similar outcomes were perceived for tomato seeds, with effects varying based on the parameters and concentrations used. The ability of active nanoparticles or nanocarrier systems to improve seed germination stems from their capacity to infiltrate the seed coat at optimal concentrations, thereby augmenting the number of permeable sites for water absorption and oxygen exchange. (Guha et al., 2021; Joshi et al., 2018). The augmentation of the biological efficacy of active compounds through nanocarrier systems is linked to improved physical-chemical properties, including enhanced solubility, protection against degradation, and increased bioavailability, which can lead to a reduction in the required concentration of the active compound and a decrease in potential toxic effects (Fraceto et al., 2016; Lombardo et al., 2019). Several investigations have indicated that seed nano-priming is more effective than traditional approaches and can help to enhanced agricultural operations. Many methods categorized as nano pesticides or nano fertilizers, employed for pest control or plant development, have the potential for utilization in nano-priming seeds (Cadena et al., 2018; Fischer et al., 2019).

1.3.4 Effect of TiO₂ NPs on Plants

TiO₂ NPs represent insoluble semiconductive materials possessing a high refractive index, UV absorption, photocatalytic, and antimicrobial properties, which introduce them as a novel generation of materials due to their distinctive characteristics (Siddiqui et al., 2019). These engineered nanoparticles (ENPs) exhibit versatile crystal symmetries such as anatase, brookite, or rutile, each with distinct properties that affect their application. Typically, the mineral form is chosen based on its lower or higher photocatalytic potential. Their size, ranging from a few nanometres to 100 nm in each dimension, and apacity to form TiO₂ nanoparticles into nanorods or spherical nanoparticles during synthesis make their properties highly tunable (Šebesta et al., 2021). Titanium dioxide nanoparticles exhibit robust oxidation reactions, particularly targeting organic compounds. For example, applying TiO₂ NPs on barley, first during stem elongation and later during the four-

leaf stage at concentrations between 0.01 and 0.03 percentages led to elevated grain yield. It increased 1000 grain weight (Moaveni et al., 2011).

TiO₂ is a widely recognized nanoparticle significantly impacting plant growth and development, being the ninth most abundant element on Earth. Therefore, it is crucial to assess both its toxic and beneficial effects on plants. These nanoparticles have diverse effects on crop morphology, biochemistry, and physiological traits (Mishra et al., 2014). Other research (Shah et al., 2021a) showed germination rates and improved radicle and plumule development in rapeseed seedlings upon TiO₂ nanoparticle treatment. Jaberzadeh et al., (2013) presented increased growth and yield in wheat seedlings under water stress due to TiO₂ NPs. TiO₂ NPs regulate enzyme activity associated with nitrogen metabolism in plants, influencing the uptake of nitrate and facilitating the conversion of inorganic N into organic forms such as chlorophyll and amino acids, ultimately boosting protein production and plant biomass (Mishra et al., 2014). Hong-Bo et al., (2005) found that TiO₂ NPs protect chloroplasts against high light intensity by increasing antioxidant enzyme activity, such as POD, SOD, and CAT. Shah et al., (2021) investigated nano-priming with TiO₂ as a strategy to alleviate salinity stress in maize, suggesting its potential as an effective method to mitigate the adverse impact of salinity stress on maize crops due to increased anti oxidant activity as mediated and affect on germination rate and seedling vigor.

1.4 History of Seed Priming

Seed priming originated in ancient Greece, where early seed treatment procedures were developed to address challenges of low germination and poor seedling growth (Sarkar et al., 2019). The initial investigations in seed science concentrated on the morphological aspects of germination (Sachs, 1859). Subsequently, technologies for treating seeds with various organic and inorganic chemicals were developed (Lutts et al., 2016).

The Greek philosopher Theophrastus (371-287 B.C.) discovered that soaking seed cucumbers in water or honey resulted in faster and more uniform germination (Theophrastus & Arthur, 1916). Ells, (1963) optimized key such as solution quantity, aeration, and treatment duration. He found that seeds treated with a salt solution exhibited quicker emergence due to the accumulation of RNA, which translated into the enzymes necessary to activate the physiological processes of seed

germination, even in stressful conditions (Berrie & Drennan, 1971), while the salt solution positively affected seed germination, some glycophyte species suffered from seed hardening due to the high salt concentration. This issue was resolved by (Heydecker et al., 1973) who introduced polyethylene glycol (PEG), a high molecular weight organic chemical, for seed treatment.

The discovery of hormones and their roles in physiological processes like drought stress, reserve mobilization, cell division, and elongation in the 1920s opened new avenues for enhancing biochemical compounds in seed treatment (Lutts et al., 2016). The publication of a report by Heydecker & Coolbear, (1977) on seed treatments aimed at breaking dormancy and improving germination paved the way for further advancements in this field. Bradford, (1986) later explained the principles of plant water relations in seed priming, while (Taylor et al., 1998) introduced the term, seed priming. Halmer, (2006) further emphasized the practical applications of seed treatment technologies.

1.5 Conventional Seed Priming

To achieve successful outcomes in commercial agriculture, it is crucial to ensure efficient, rapid, and uniform seeds germination and the establishment of seedlings. In smart agriculture, another promising nanotechnology-based approach has emerged, known as seed priming. This technique aims to enhance seed quality, accelerate germination, and promote plant growth, contributing to improved agricultural efficiency. A pre-sowing technique that induces physiological changes in seeds, resulting in accelerated germination known as seed priming (Bruce et al., 2007). It also boosts crop activity by increasing plant resilience to abiotic and biotic stressors (Arnott et al., 2021). Before planting, seeds are treated through a priming process. This includes conventional techniques like soaking beforehand and applying coatings. During the pre-germination stage hydration techniques, with or without aeration, are applied to the seeds. After this stage, the seeds are brought back to their standard moisture content for regular handling processes like sowing, packaging, and storage. Priming primarily supports seed germination across three distinct stages, namely imbibition, germination, and growth (see Figure 1.2). In the imbibition stages, the absorption of water facilitates processes like protein synthesis and respiration through messenger ribonucleic acid (mRNA). The subsequent phase is characterized by the initiation of several physiological activities related to germination, including as mitochondria formation, protein synthesis, and

alterations in soluble sugars (Varier et al., 2010). A critical feature of seed priming is the controlled water intake in the second phase, which occurs before the emergence and growth of seedling roots from the seed coat in the final phase. Notably, the second phase (germination) is far more vulnerable to environmental stimuli than the third phase (Côme & Thévenot, 1982). As a result, seeds that have passed through the second phase of priming may germinate under different environmental conditions than unprimed seeds (Corbineau & Come, 2006).

Hydro-priming, osmo-priming, nutritional priming, chemical priming, bio-priming, seed priming with plant growth regulators, and priming with plant extracts are the conventional methods.

Hydro-priming, a cost-effective technique, involves soaking seeds in water for a specific period and then drying them to a predetermined moisture level before sowing (H. Singh et al., 2015; U. Singh et al., 2015). This method is particularly beneficial in regions facing challenging environmental conditions, such as high temperatures and drought stress. Hydro-priming enhances water uptake efficiency and seed hydration, which is especially advantageous under adverse conditions (Black & Bewley, 2000). However, it's crucial to carefully control humidity and temperature to prevent premature radical emergence, as uncontrolled water uptake can occur (Taylor et al., 1998). A key aspect of hydro-priming is drum priming, originally developed by Rowse (1991). It utilizes a drum filled with seeds connected to a vapor-producing boiler. The vapor condenses into water droplets inside the drum, with precise monitoring of water volume and hydration time (Warren & Bennett, 1997). Research indicates that hydro-priming significantly enhances germination and seedling growth, particularly during drought stress conditions, with improvements of up to three to four times compared to non-primed seeds (Kaur et al., 2002). This method has proven to be successful through various crops, including both food crops and desert plant species, facilitating uniform and early germination.

Osmo-priming is a method involving the soaking of seeds in a solution containing substances like mannitol, sorbitol, or osmotic agents such as polyethylene glycol, as detailed by (Sher et al., 2019). Conversely, when seeds are soaked in inorganic salt solutions, like sodium chloride, calcium chloride, and calcium sulfate, to enhance establishment and seedling growth, it's referred to as halopriming, also explained by Sher et al., (2019) The duration of the priming

process, typically ranging from -1.0 to -2.0 MPa, varies depending on the plant species, spanning from 1 to 2 days for lettuce, sorghum, and rice, up to 5-7 days for tomato and sugar beet, and between 7-10 days for cauliflower, carrot, fennel, primrose, and pansy. For celery and leek, it extends up to 14 days. After priming, seeds are generally dried back to their usual moisture content before storage and sowing (Bino et al., 1992; Özbingöl et al., 1999; Saracco et al., 1995).

In a study by Jalali & Salehi, (2013), sugar beet seeds were subjected to treatment with PEG 8000, sodium chloride 1.5 (N), and chloride hydrogen 0.1 (N) for 2, 4, and 6 hours. It was observed that chloride hydrogen at 0.1 (N) treatment for approximately 6 hours had a substantial impact on seed characteristics. In this case, the germination percentage was significantly higher, by 25 and 9 percent, compared to control and sodium chloride with 1.5 (N) treatments after the same duration. Seed priming with PEG 8000 and sodium chloride at 1.5 (N) showed moderate and low effects, respectively.

Beata et al., (2018) explored the impact of solid matrix priming on sugar beet (cv. Janosik) cultivation, a diploid cultivar with monogerm characteristics known for its resistance to rhizomania. This method led to faster and more uniform emergence of primed seeds, subsequently resulting in a significant increase in mean root mass during harvest. However, it did not affect final plant density or root yield. Similarly, Chomontowski et al., (2020) reported the effectiveness of seed priming for sugar beet, focusing on seed quality and environmental conditions. Their research, conducted over three years using two sugar beet seed varieties with varying vigor, employed patented solid matrix priming methods in both phytotron and field settings. The results showed that primed seeds with higher vigor exhibited accelerated leaf development and increased plant dry matter throughout the growth season, leading to a significant boost in sugar yield. Priming was also found to reduce seed loss during treatment and minimize variations in germination and emergence caused by changing seed vigor and germination conditions.

Studies utilizing flow cytometry have shown that osmo-priming of tomato seeds activates DNA replication in radicle tip cells, with an increase in 4C DNA content also similarly observed in sugar beet seeds (Redfearn & Osborne, 1997). Additionally, Job et al., (1997) indicated that an 11-S globulin beta-chain serves as a valuable biomarker for detecting the impact of seed priming in sugar beet. The study found an increase in α -glucosidase accumulation, leading to increased levels

of globulin breakdown. Other researchers (Ali et al., 2019) and (Capron et al., 2000) found comparable sugar beet seed priming results, demonstrating higher mobilization of the 11S-globulin- β -subunit.

Moreover, phytohormones, including jasmonic acid, cytokinin, gibberellin, auxin, salicylic acid, abscisic acid, ethylene, brassinosteroid, and others, have a crucial role in plant growth and development. Therefore, hormonal priming involves the use of phytohormones or plant growth regulators to enhance crop performance in various growing conditions. Ullah et al., (2019) discuss how seed priming with different concentrations of tryptophan in sugar beet resulted in increased growth, yield, and root quality. It also mitigated the negative effects of salinity stress by promoting higher chlorophyll content and carotenoid levels. Additionally, nutrient priming is a strategy that involves soaking seeds in micro or macronutrients at specific concentrations, like nitrogen, phosphorus, or potassium, to enhance water regulation during germination for improved growth, as detailed by Shivay et al., (2016). It is notable that primed seeds demonstrate more favorable cell membrane performance and structure compared to regular control seeds, indicated by lower electrical conductivity in the seed extract. This effect has been shown in primed seeds from several plants, including sweet corn.

1.6 Impact of Nanotechnology on Seed Priming

Plant responses to various abiotic stresses are primarily influenced by factors such as the severity of stress, the duration of exposure, and plant growth level (Ding & Yang, 2022; Rivero et al., 2022; Zandalinas et al., 2021). Adverse conditions can have varying effects on plant performance at different phenological stages, as can the exogenous use of plant modulators to mitigate the negative consequences of stress.

Diverse forms of nanoparticles, including Ag and ZnO, activate antioxidant defense systems in plants, reducing ROS levels and promoting growth (Acharya et al., 2020; Waqas Mazhar et al., 2022). Research has shown that seed nano-priming is more effective at enhancing germination and seedling development than other methods of priming that involve ionic and bulk materials (Abbasi Khalaki et al., 2021; do Espirito Santo Pereira et al., 2021; Kandhol et al., 2022). NPs can break seed dormancy by increasing food and water intake, activating enzymes containing CAT, proteases, SOD, and amylases, likewise improving plant defense systems in response to environmental challenges (Acharya et al., 2020; Mahakham et al., 2017;

Sz\Holl\Hosi et al., 2020). This process is expected to enhance seed vigor, which is crucial for subsequent seedling development. Moreover, Seed nano-priming offers the potential for seed protection, as numerous nanoparticles possess antimicrobial properties and can even carry antimicrobial agents (Abbasi Khalaki et al., 2021; Pirzada et al., 2020).

Applying NPs, mainly through nano-priming and foliar exposure, modulates the molecular processes of seed germination, seedling growth, and plant maturation. The advantages of seed nano-priming can be linked to alterations caused by the interaction of nanoparticles with seed metabolism, various physiological and biochemical processes, and signal pathways within seeds. These changes include the regulation of ROS balance and their interaction with phytohormones, as supported by several studies (Abbasi Khalaki et al., 2021; Abdel Latef et al., 2018; do Espirito Santo Pereira et al., 2021; Faizan et al., 2018; Shang et al., 2019; Tripathi et al., 2017). However, there is a need for further research on the mechanisms underlying the interactions between nanoparticles and different plant reproductive stages. Such studies should encompass various omics analyses and consider phenotypic and agronomic aspects of plant tolerance to a range of abiotic stresses.

A notable distinction exists between seed priming and seed nano-priming. Traditional seed priming typically involves the use of water (hydro-priming) or solutions containing various substances such as hormones, nutrients, or biopolymers, which adhere to the seed surface and can lead to seed coating. In contrast, seed nano-priming employs suspensions or nanoformulations as the medium, and these contain nanoparticles that may or may not be absorbed by the seeds. Even when seeds do absorb nanoparticles, many of them remain on the seed's surface, forming a coating. This protective layer, as described by (Acharya et al., 2019; Falsini et al., 2019) be combined with fungicides or bactericides to safeguard seeds from diseases either in the field or during storage (Gross et al., 2020).

1.7 Studying on the Physiology and Biochemistry of Nano-primed Seeds

1.7.1 Germination of Seed

Germination, a crucial step in agriculture, plays a critical role in ensuring the quality of crops. It promotes the rapid development of seedlings, enabling them to expand their leaves and elongate their roots quickly. This, in turn, increases

nutrient uptake, their movement through transpiration, and biomass production. Conversely, a delay in the germination period can make young seedlings vulnerable to the plant life cycle, environmental stressors, and pathogens. This vulnerability can lead to reduced vigor and crop productivity, ultimately causing financial losses for farmers (Acharya et al., 2019, 2020; Mahakham et al., 2016).

The process of seed germination is commonly categorized into three distinct phases (Nonogaki, 2014; Nonogaki et al., 2010). It is activated with imbibition (phase I), characterized by the rapid absorption of water which initiates metabolic activities, including protein synthesis, transcription, and mitochondrial function. In phase II (referred to as activation or lag phase), water intake slows down, but the seed metabolism is intensified, resulting in the production of enzymes necessary for mobilizing reserves and developing the embryo. These enzymes include amylases, endoxylanase, and phytase. In phase III, there is another increase in water absorption, which leads to the peak of embryo development, particularly the emergence of the radicle (Ibrahim, 2016; Nonogaki et al., 2010).

The nano-priming technique can control the metabolism of seeds stimulating the metabolic reactions that usually start in the early stages of germination. This process enhances seed germination by promoting various aspects, including increased water uptake, accelerated starch hydrolysis, loosening the cell wall, weakening the endosperm, promoting rapid embryo development, and increasing root and shoot growth (García-Gómez & Fernández, 2019).

While a complete understanding of the signaling and phytohormones cross-talk during germination remains elusive, researchers have observed the importance of the balance between abscisic acid (ABA) and gibberellic acid (GA) in regulating water uptake by altering the water potential threshold during germination (del Carmen Rodríguez-Gacio et al., 2009). Additionally, ABA plays a role in controlling genes from the aquaporin family, such as PIP2, NIP1, TIP3, and TIP4, during seed germination (Footitt et al., 2019). As observed by Watahiki & Yamamoto, (1997) the role of auxin in the adhesion and transport of nanoparticles to the seed coat and endosperm is evident in the *msg1*-like insensitive mutant. This is particularly important for hypocotyl elongation and is affected by both auxin and ABA.

1.7.2 Malondialdehyde (MDA)

The presence of MDA, a byproduct resulting from the oxidation of polyunsaturated fatty acids, serves as a valuable indicator of overall lipid peroxidation. MDA or thiobarbituric acid reactive-substances (TBARS) assay has been widely employed since the 1950s for estimating lipid peroxidation in membrane and biological systems (Hodges et al., 1999). Malondialdehyde is generated through the autooxidation and enzymic degradation of polyunsaturated fatty acids within cells.

Additionally, ROS are vital, and perform both as catalysts for the germination process (as signaling molecules) and as potential sources of oxidative damage (cytotoxic effects). Detection and quantification of ROS are critical procedures in seed quality assessment, especially in seed priming (Steffens et al., 2013). Indirect assessment requires the quantification of lipid peroxidation, indicated by MDA content, in both dry and soaked seeds. This method has been used in research to investigate the effects of seed priming on different plant species, such as sunflower (Bailly et al., 2000), cucumber (Krainart et al., 2015), barley (Junhaeng et al., 2018), wheat (Hameed et al., 2019), rice (Sen & Puthur, 2020), and *Solanum villosum* (Forti et al., 2021).

In research conducted by Naguib & Abdalla, (2019) nano-silica was used to prime *Zea mays* seedlings under salinity stress. Primed seeds showed a decline in the accumulation of H₂O₂ and MDA, as well as a boost in the antioxidant activity of SOD and POX compared to the non-primed seeds. However, it's crucial to consider factors such as the specific type of nanoparticles, the plant species involved, and the current growing conditions when considering their application in priming.

1.7.3 Effect on Photosynthesis

The process by which higher plants, algae, and particular microbes absorb sunlight and transform it into organic compounds with a high energy content that is subsequently stored is known as photosynthesis. Nano-priming has provided insights into the photosynthetic mechanism of plants (Acharya et al., 2020).

The supplementation of nanomaterials on pepper leaves, such as foliar sprayed CuO NPs, ZnO NPs, MgH NPs, and MgO NPs at appropriate concentrations, play a role as fertilizer, surpassing control conditions by increasing chlorophyll content, plant height, and leaf growth (Ghidan et al., 2018).

The priming of Egyptian roselle (*Hibiscus sabdarifa* L.) cultivars with a lower concentration (0.01%) of aluminum oxide (Al_2O_3) NPs significantly enhanced the growth parameters of leaf surface, shoot, and root length, fresh and dry mass alongside improvements in physiological and biochemical functions. These included increased levels of carotenoid, chlorophyll a and b, soluble sugars, protein, proline, and the efficiency of various antioxidant enzymes such as SOD, CAT, POD, and APX. This suggests that seed priming in the Egyptian roselle cultivar can produce positive effects even at lower concentrations (Abdel Latef et al., 2018).

In wheat, applying TiO_2 NPs in the soil increases tolerance to drought by raising osmolyte content, root and shoot lengths, membrane stability, total chlorophyll, and fresh and dry weights (Mustafa et al., 2021). Under the water deficiency stress, the applying TiO_2 NPs at the 4-6 leaf stage and after that for two weeks enhanced drought resistance and maize grain yield by amplifying leaf antioxidant activities SOD, APX, and CAT while also increasing leaf proline content (Karvar et al., 2022). Similarly, exogenous treatment with nitric oxide increased the defensive impacts of TiO_2 NPs on wheat seedlings under water deficit stress, improving plant growth, photosynthetic performance, and the antioxidant system (Faraji & Sepehri, 2020).

1.7.4 Starch Metabolism

α -Amylase, an enzymatic catalyst, operates hydrolytically, breaking down reserved carbohydrates into soluble sugars. This process sustains an active respiratory metabolism crucial for seed germination and early plant growth, preceding the initiation of photosynthesis (Kohno & Nanmori, 1992). The increase in α -amylase activity, which stimulates rapid starch breakdown in germinating seeds treated with nano-priming, potentially contributes to increased germination rate and vigor in seedlings (Mahakham et al., 2017). Increasing the concentration of sugars in cells reduces osmotic and water potential, causing seedling growth and acceleration of germination (Mahakham et al., 2017). Observations by Man et al. indicate that α -amylase activity initially affects the surface of starch granules, subsequently leading to protrusion inward and outward which indicates an increase in α -amylase activity in seeds treated with the nano-priming technique (Man et al., 2013). Moreover, the induction of α -amylase production depended on gibberellic

acid (GA) activity. For example it indicated that the link between the absence of α -amylase biosynthesis and the deficiency of GA (Mahakham et al., 2017). SiO₂ NPs treatment in wheat increased drought tolerance by improving seed germination, seedling growth, antioxidative defense system, amylase activity, and water uptake efficiency (Akhtar et al., 2021).

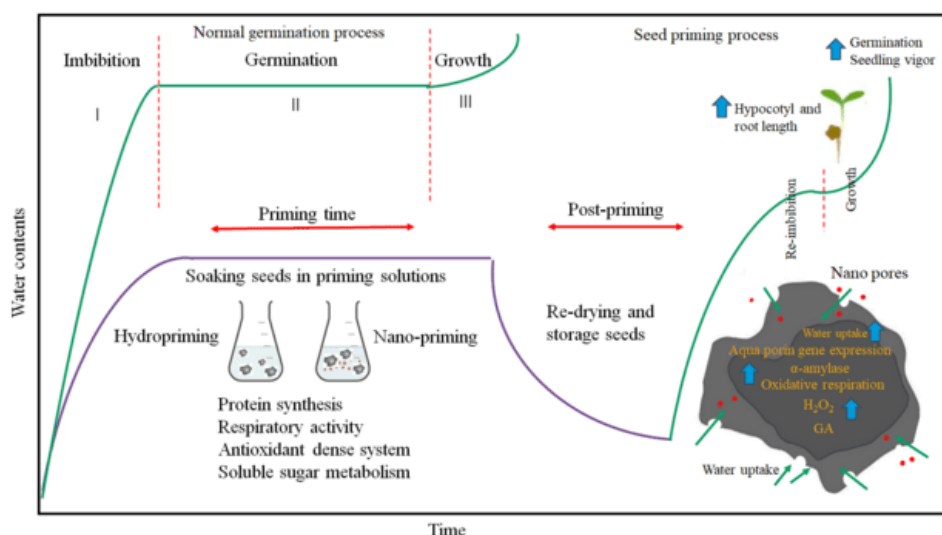


Figure 1.2. Comparison germination process in normal and seed priming condition.

Totally, the seed priming process involves the partial imbibition of seeds until germination (phase II) begins but is suspended before radicle growth is complete. During priming, the moisture content of the seeds is kept at 40-45% of their fresh weight, which is approximately 90-95% of what is required for radicle growth. Controlled water absorption during the first phase (imbibition) promotes protein synthesis and increases respiratory activity. Phase II includes physiological, metabolic, and molecular functions such as protein synthesis, respiration, soluble sugar metabolism, and repair mechanisms, but radicle formation is purposely avoided. Subsequently, upon sowing, the germination rate of primed seeds improves (Figure 1.2).

2. AIM AND SCOPE OF THE STUDY

The main objectives of this thesis investigation were outlined as follows:

- (i) To examine the impact of the seed priming technique in sugar beet seed,
- (ii) To compare “hydro-priming” as the traditional method to “nano-priming” as an advanced method,
- (iii) To understand the “priming time” effects on the synchronized germination and vigor of sugar beet seeds,
- (iv) To estimate whether "post-priming" could contribute to the preserved properties of this approach into seed to improve seed quality by mitigating the detrimental impacts of environmental stress conditions on sugar beet plants,
- (v) To investigate the biochemical and physiological processes of sugar beet seeds and succeeding growth and development.

Hence, it is anticipated that these findings will aid agricultural research efforts focused on enhancing the yield and quality of sugar beet seeds; this technology will lead to the development of the industry of sugar.

3. MATERIALS AND METHODS

This research was carried out at the Plant Biotechnology Laboratory in the biology department of Bolu Abant Izzet Baysal University.

3.1 Plant Material

In all of the experiments in this study, *Beta vulgaris* L. seeds from the Chenopodiaceae family with monogerm SG188 genotype (developed by Songül GÜREL), diploid, rhizomania disease resistant, high root yield, and high sugar content was provided from Sugar Institute, Ankara, Turkey. After measuring the sample's mean dry weight of 100 grams, or approximately 0.815 grams, dry seeds (moisture content <12%; dry weight) were placed in cotton bags and stored at room temperature until required.

All experiments have been utilized under the seed priming technique information about sterilization, hydro-priming, nano-priming, germination and growing seedlings, to be used for all analyses is described below.

3.2 Nanoparticles (NPs) Used for Sugar Beet Seed Priming

3.2.1 Chemicals

The impacts of TiO₂ synthetic metal on seedling germination, growth, and advancement as a nano-priming technique have been considered. Commercial grade mentioned nanopowder TiO₂ (NPs size <150 nm (DLS), CAS No. 13463-67-7) was provided by Sigma Chemical Co. (St Louis, MO, USA).

3.2.2 Preparation and Characterization of the Nanoparticles

Characterization of the nanoparticle titanium dioxide (TiO₂) has been chosen according to its characteristics. The synthesized NPs were employed without changes and poured into double distilled water to achieve the appropriate concentration. To obtain a well-mixed distribution and reduce aggregation, the NPs solution was stirred and ultrasonically sonicated before being applied to the nanoprimer solution.

In the study of Dipul, K.B. (2024), TiO₂ nanoparticles were evaluated assessment through various analytical techniques. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray powder diffraction (XRD) were used to measure crystalline sizes.

3.3 Seed Priming Technique

3.3.1 Determination of Moisture Content in Seeds

The moisture content assessment of sugar beet seeds followed the Constant Temperature oven method outlined by the International Seed Testing Association (ISTA, 2021). For this process, glass petri dishes measuring 8 cm in diameter were utilized as drying containers. The petri dishes were initially weighed empty, after which their lids were labeled. Two replicates, each weighing 5.0 g, were extracted from the seed lots, thoroughly mixed, and homogenized, and evenly distributed into the numbered and pre-weighed glass petri dishes. Subsequently, these dishes were sealed with their lids and re-weighed with the samples enclosed. To measure the moisture contained in sugar beet seeds, the sealed glass petri dishes were placed in an oven preheated to $130\pm 2^{\circ}\text{C}$ and kept there for 1 hour. After this duration, the petri dishes were taken from the oven while still sealed and allowed to cool to room temperature in a desiccator containing silica gel. After cooling, the petri dishes with the samples were re-weighed. The data collected were assessed using the formula specified by ISTA guidelines (ISTA, 2021).

$$\text{Seed Moisture Content (\%)} = [(M2 - M3) / (M2 - M1)] \times 100$$

M1: Weight of empty petri dish with label and lid (g)

M2: Weight of seeds before drying + petri dish with lid (g)

M3: Weight of seeds after drying + petri dish with lid (g)

During the analysis of seed moisture content, it was ensured that the maximum variance between the two replicates remained below 0.2%. Otherwise, the assessment of seed moisture content was conducted again. The original moisture contents of sugar beet seeds were determined as 5.06% for the first sample and 5% for the other one.

The final moisture contents after priming treatments in sugar beet seeds were estimated according to the following formula (Sivritepe, 1992):

$$d = 100 - [a (100 - b) / c]$$

d: Final moisture content of the seed (%)

a: Initial weight of the seed (g)

b: Initial moisture content of the seed (%)

c: Final weight of the seed (g)

3.3.2 Seed Sterilization Followed by Hydro-Priming and Nano - Priming Solutions Exposure

Sugar beet seeds were randomly separated from the seed mass of the sugar beet monogerm cultivar which included 26 masses, inside of every mass contained 100 seeds. All masses were surface sterilized by washing tap water for 4 hours at 25-27 °C temperature and then was soaked in tyramine carboxy (2000 ppm) solution for 30 minutes as disinfection. After sterilization sugar beet seeds, were kept in an oven device to dry for 24 hours at room temperature (RT). Solutions containing various concentrations of nanoparticle (NPs) TiO₂ (5, 10, 15 ppm) were used for nano-priming and water as hydro-priming. For priming dried seeds and transparent cylindrical containers with 500 ml distilled water were used and one bag of un-primed seeds kept as control in experiment. In each container, bags of sugar beet seeds were placed, and during the priming, the seeds were also simultaneously aerated by an air pump device. To apply different priming durations, the first sample bag containing seeds was put in a solution containing nanomaterial and water with shaking for 3 hours, after 6 hours, another 9 hours of treatments were removed from each of the solutions. Then, primed seed bags were washed with water for 20 minutes three times. Seeds were re-dried by monolayer spreading on blotting papers for 48 hours at RT in an oven device. Seeds were put into suitable bags after re-drying and their lids were punched. All specifications of the sample were recorded on the packages as post priming (10 and 45 days) and placed in the dark condition at a temperature of about 4°C till used for germination. A total of three independent biological experiments were performed under the same growth conditions.

3.3.3 Evaluation of Germination and Growth Parameters

10 and 45 days after priming, primed seeds were sown in order to measure the desired traits including germination percentage, root length, shoot length and biomass. The seeds were counted and planted on pleated filter papers in white boxes. After adding 40 ml of water to the pleated filter papers, the moistened filter papers with the seeds were placed in a chamber room at a temperature of $23 \pm 1^{\circ}\text{C}$, under cool-white fluorescent light with an irradiance of $50\mu\text{mol m}^{-2} \text{s}^{-1}$, and kept at a relative humidity of 55–60% until they were 10 days old. Germination rates were determined by calculating the quantity of germinated seeds (measured as 2 mm radicle emergence) at 24-hour intervals. The seed

germination rate was calculated by dividing the number of germinated seeds by the total number of seeds used in the germination experiment. And, the formula is used for the calculation of the rates;(Awasthi et al., 2016)

$$GR = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \dots + \frac{\text{Number of germinated seeds}}{\text{Day of final count}}$$

The germination rates of the primed seeds were then compared to those of the control group by analyzing their respective percentages. The results were collected based on data from three replications and presented as mean values along with their respective standard deviations.

3.3.4 Determination of Developmental Phytotoxicity of Seed Germination

The effect of seed germination on plant growth was evaluated by a comparison of treatment and control groups. Parameters including germination percentage, shoot and root lengths, as well as seedling vigor, were quantified and subjected to analysis. Statistical methods were employed to identify any significant variances among the different treatment groups. The germination rate was computed by dividing the count of germinated seeds by the total number of seeds utilized in the germination trial (EPA, 1996).

$$\text{Percentage (\%)} \text{ of seed germination (GP)} = [(gC - gT) / gC] \times 100$$

where gC is the number of seeds germinated in the control group and gT is the number of seeds germinated in the treatment group.

According to ISTA (1996) all treatments and control group were monitored for germination (2 mm of radical long) , the end f germination period (10 days) from every treatment and each replicate choosen 5 seedlings randomly to calculated the seedling vigor index according this formula: (Shokohian & Omidi, 2021)

$$\text{Seedling vigor index (SVI)} = \text{GP} \times \text{Mean (SL)}$$

GP is germination percentage; SL is seedling length (shoot length+root length)

3.3.5 Morphological Assessment

Morphological assessment of seeds primed and unprimed was monitored under the stereomicroscope (Olympus SZ51) and during germination, photography

was conducted to monitor and compare any differences in the appearance of the treated plants as part of the morphological assessment.

3.3.6 Determination of Seedling Dry Weight, Length of the Roots, and Hypocotyls

On the 10th day of seedling growth, five seedlings were randomly chosen from each box and measured for plant height and fresh weight. After the experiment, the plants were subjected to an oven at a temperature of 70±1°C for 24 hours to measure their dry weight. Moisture content is commonly expressed as a percentage relative to the initial sample weight, often referred to as fresh weight (Hay et al., 2023).

The water content in a sample of seeds is typically quantified as a percentage of the initial (fresh) weight of the sample:

$$MC (\% \text{ f. wt.}) = 100 \times \frac{\text{Starting sample weight} - \text{dry weight}}{\text{Starting sample weight}}$$

The influence of nanoparticles on plant growth parameters was assessed by measuring root and hypocotyl length using the ImageJ program (National Institute of Health Software).

3.3.7 Physiological and Biochemical Assessment of Seed Priming Technique

3.3.7.1 Quantification of Alpha-amylase

The materials were sourced from identical lots of both primed and control seeds, which were utilized in determining the soluble sugar content. A total of 150 true seeds were meticulously selected from each treatment group using tweezers, then ground in a mortar with liquid nitrogen. Crude enzymes were extracted by the method of Lawrence et al., (1990). 30 milligrams of the ground material were homogenized in 1.5 mL PBS on ice and sonicated for 30 seconds using a microprobe (Vibra-Cell™ Ultrasonic Liquid Processors- VC50). Then the obtained homogenates were centrifuged for 10 minutes at 2000 g (MPW MED, Instrument), and the supernatants were utilized for subsequent analyses.

Amylase activity was evaluated using the starch-iodine approach with potato soluble starch as the substrate. The amylase assay comprised 300 µL of 1% (w/v) soluble starch from potato, 450 µL of CH₃COOH/CH₃COONa (pH 4.5) buffer, 50 µL of 0.1 M CaCl₂, and 200 µL of seed extract. This mixture was

incubated at 37°C, with 200 µL aliquots collected at 0 and 5 minutes after the start of incubation. The reaction was halted by adding 200 µL of 1% (w/v) KI+O.1% (w/v) I₂, and then the solution was diluted 8-times with distilled water to determine the absorbance at 620 nm. One unit of amylase activity was defined as a 10% reduction in absorbance at 620 nm per minute.

3.3.7.2 Quantification of Chlorophyll Pigment

To evaluate the amount of chlorophyll, 100 mg of leaves were ground using a mortar and pestle with liquid nitrogen. After that, they were homogenized in 1 ml of 100% dimethylformamide (DMF) using a sonicator device for approximately 1 minute. Then, the tubes were incubated for around 15 minutes at 4°C. Following this, centrifugation was performed for 10 minutes at 27,000 ×g, and the supernatant was transferred to a new tube. The samples were analyzed using a UV–visible spectrophotometer (Hitachi Model U-2000), and absorbance was measured at wavelengths of 664.5 nm and 647 nm. Chlorophyll a and chlorophyll b contents, and consequently total chlorophyll, were quantified using the formula (Moran, 1982):

$$\text{Chl a} = (12.92 A_{664.5}) - (2.12 A_{647}) - (3.85 A_{603})$$

$$\text{Chl b} = (-4.67 A_{664.5}) + (26.09 A_{647}) - (12.79 A_{603})$$

$$\text{Total} = (8.24 A_{664.5}) + (23.97 A_{647}) - (16.64 A_{603}).$$

3.3.7.3 Quantification of Malondialdehyd (MDA)

According to the method of Zhang et al., (2012) The assessment of malondialdehyde (MDA) content was done as follows: each leaf tissue sample weighing 100 mg was turned into powder using liquid nitrogen. After filling the tubes with powder, 1 ml of 0.1% (w/v) trichloroacetic acid (TCA) was added and mixed gently inverted to ensure homogeneity of the leaf tissue. The homogenized samples were then centrifuged at 10,000 x g for 10 minutes, and the resulting supernatants were delicately transferred to fresh tubes. To these supernatants, 4 ml of 20% TCA with 0.5% thiobarbituric acid (TBA) was added and carefully mixed. Then, the samples were heated to boiling at 95°C for 15 minutes and promptly cooled on ice (TBA reacted with MDA and formed a red compound). Following this, the samples were centrifuged at 10,000 x g for 5 minutes, and the resulting supernatant was transferred to fresh tubes. MDA content was determined by

measuring the absorbance of the resultant red compound using a spectrophotometer at a wavelength of 532 nm.

3.4 Statistical Analysis

One-way analysis of variance (ANOVA) was performed using GraphPad Prism software (version 10.1.0 (316)). Significant differences were tested and means were compared at a 5% probability level ($P \leq 0.05$) using Tukey's test. The results are presented as means $\pm$ standard error of the mean.



4. RESULTS

4.1 Priming with TiO₂ NPs and Hydro-priming Treatments

Morphological seed germination characteristics were observed using a stereo microscope (Figure 4.1). The germination appeared after re-drying of seed priming at the highest priming time at 9 hours (Figure 4.1, C, G and O) and rarely presented in primed seeds for 6 hours (Figure 4.1, J and N). But the seeds treated with water and titanium dioxide nanoparticles in different concentrations for 3 hours had not shown any germination before cultivation (Figure 4.1, A, E, I and M).

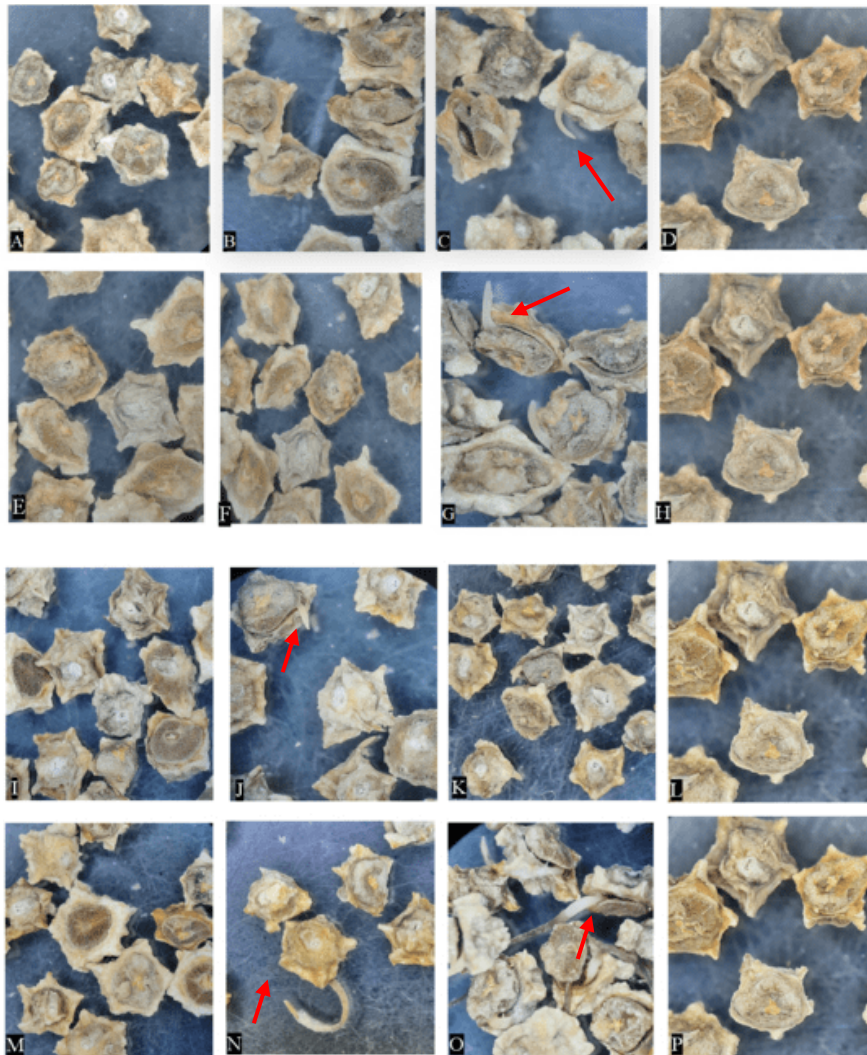


Figure 4.1. Streomicroscope pictures of primed seeds and control group after re-drying level about germination condition before cultivation. Control goroup (D, H, L, P). Hydro-priming 3,6,9 hrs showed A, B, C figures, respectively. Nano-priming with TiO₂ NPs with 5 ppm concentration for 3,6,9 hrs (E, F, G). Nano-priming with

TiO₂ NPs with 10 ppm concentration for 3,6,9 hrs (I, J, K), Nano-priming with TiO₂ NPs with 15 ppm concentration for 3,6,9 (M, N, O, P). Germinations showed with arrows in primed seeds (C, G, J, N, O).

4.1.1 The Effects of Priming on Seed Germination

The findings indicated germination-related parameters, including the germination percentage and germination rate among the different concentrations of NPs as nano-priming and another type of priming as hydro-priming that were considered in 3hrs, 6hrs, and 9 hrs as priming-time. Moreover, they were assessed as post-priming after 10 days and 45 days of priming due to a comparison of physiological and chemical features.



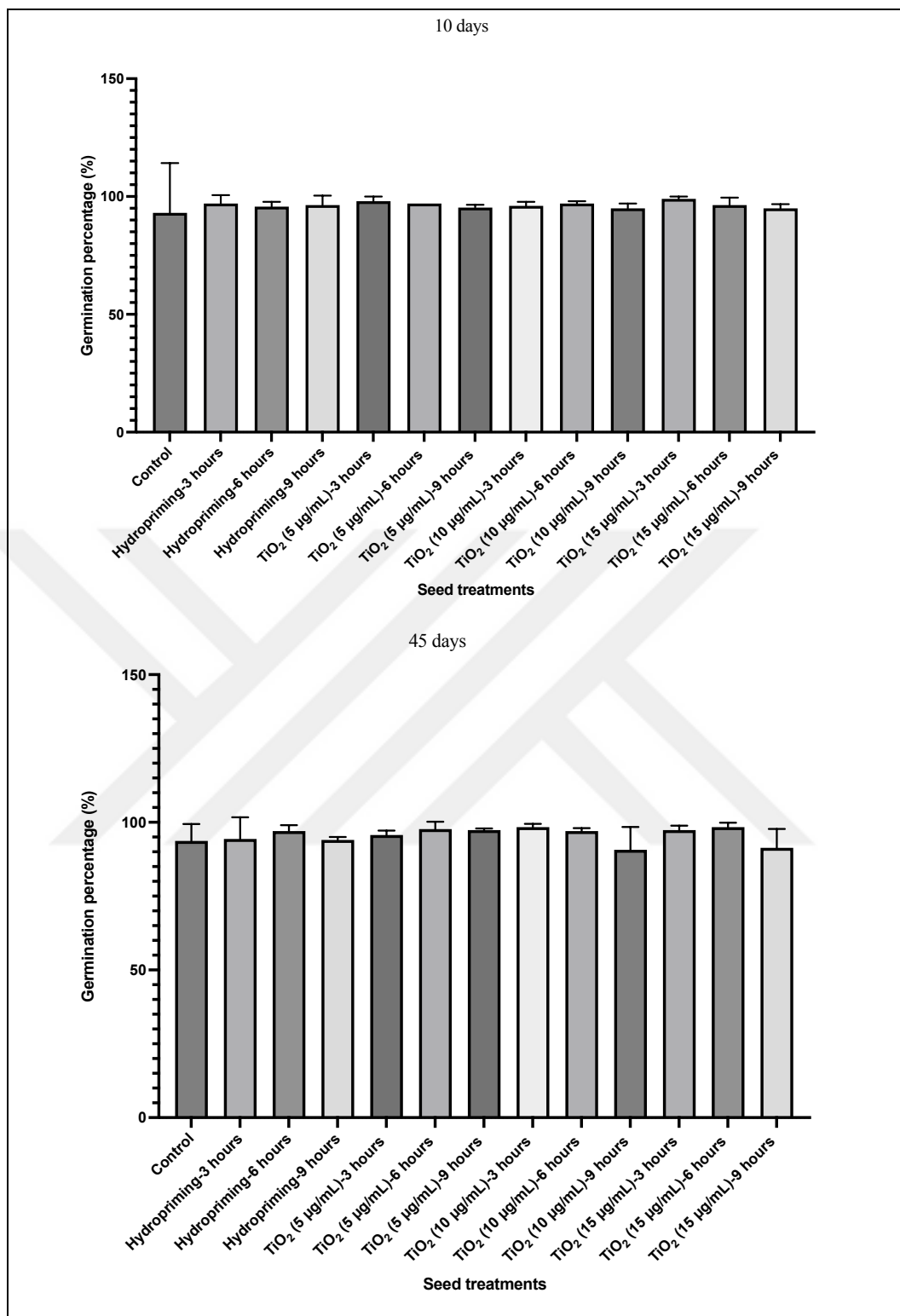


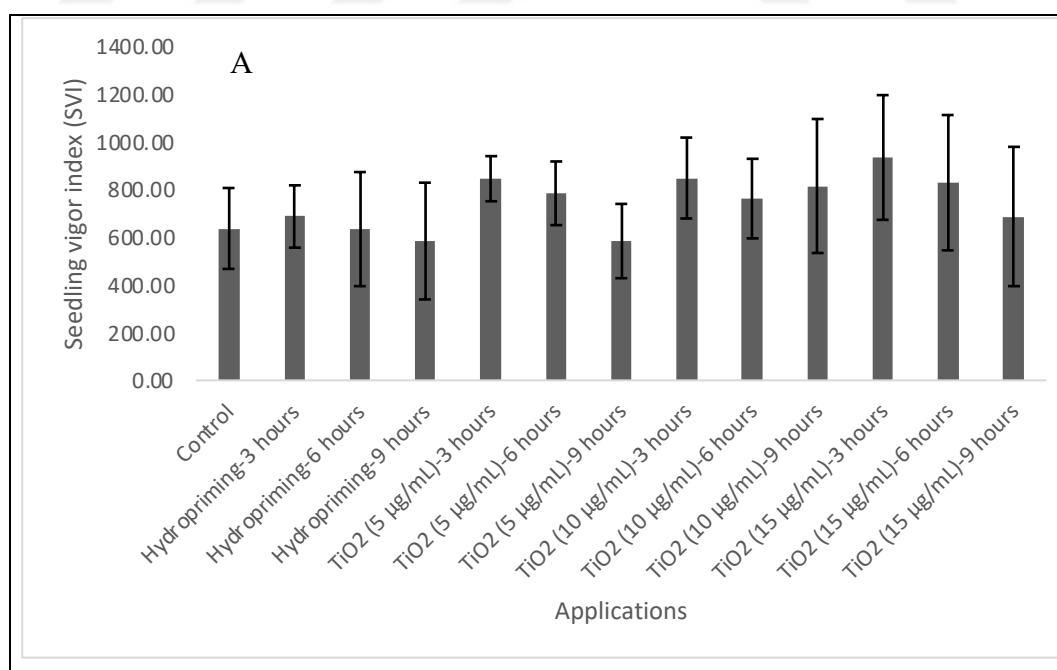
Figure 4.2. Statistical analysis of germination percentage (%) of control and priming treatments through one-way ANOVA and the results indicate no substantial differences ($P < 0.05$) among various treatments in post-priming 10 days and 45 days.

The SG188 sugar beet genotype, which was studied for nano-priming using titanium dioxide, exhibited an effect on the germination process like hydro-priming,

with germination rates comparable to the control group, as shown in Figure 4.2. For 10 days post-priming treatment, germination percentage of control group is 93.33 ± 21.16 . On the other hand, the results for the other priming conditions with different concentrations of TiO_2 and hydropriming there are no actual differentiation from the control group. The results fluctuated from 93.33 ± 21.16 (control group) to 99.00 ± 1.000 (TiO_2 NP 15 ppm, 3 hrs priming). For 45 days post-priming the same situation appears, and the result range is between 93.67 ± 5.774 for control group and 98.33 ± 1.155 for TiO_2 , 10 ppm, 3 hrs priming treatment.

4.1.2 The Effect of Seed Priming on Seedling Growth Under the Post-Priming Treatment in 10 Days and 45 Days

The average measurements of sugar beet seedling parameters include seedling length, and seedling vigor index (SVI), were significantly affected by soaking seed treatments in different substances for TiO_2 15 ppm for 3 hours, 10 days post-priming (937.23 ± 260.28). While for post-priming 45 days treatment the highest SVI belonged to substances included distilled water with 3 hours (819.73 ± 153.89) and among nano-primed treatments, TiO_2 (15 ppm) 3 hours was higher than others (Figure 4.3-A, B, Tables 4-1 and 4-2).



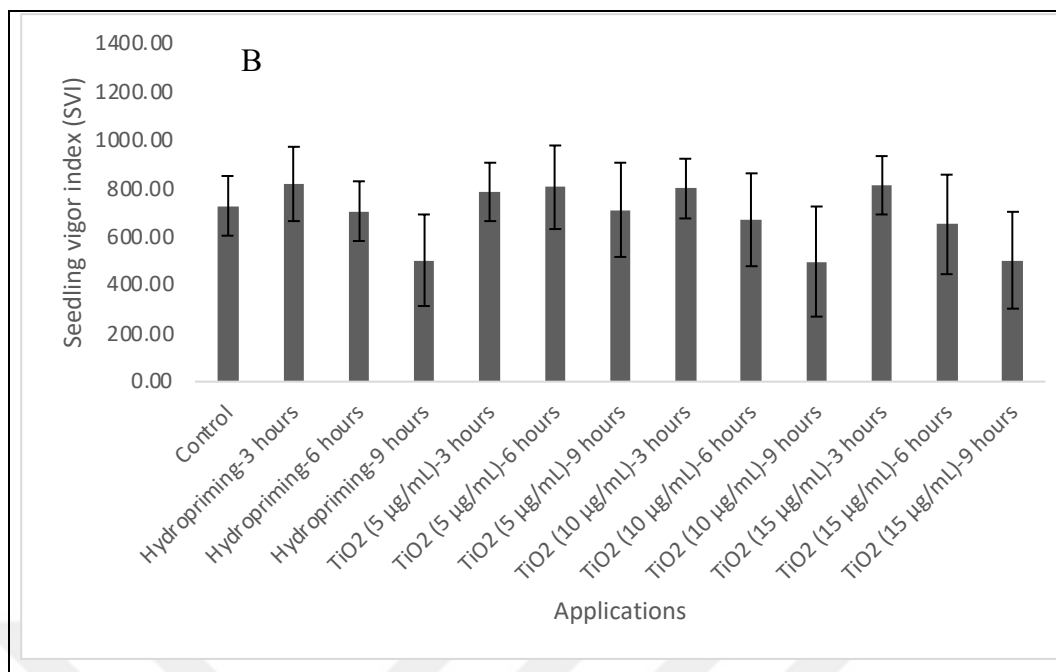


Figure 4.3. Seedling vigor indeces (SVI) **A.** for 10 days post priming, **B.** for 45 days post priming.

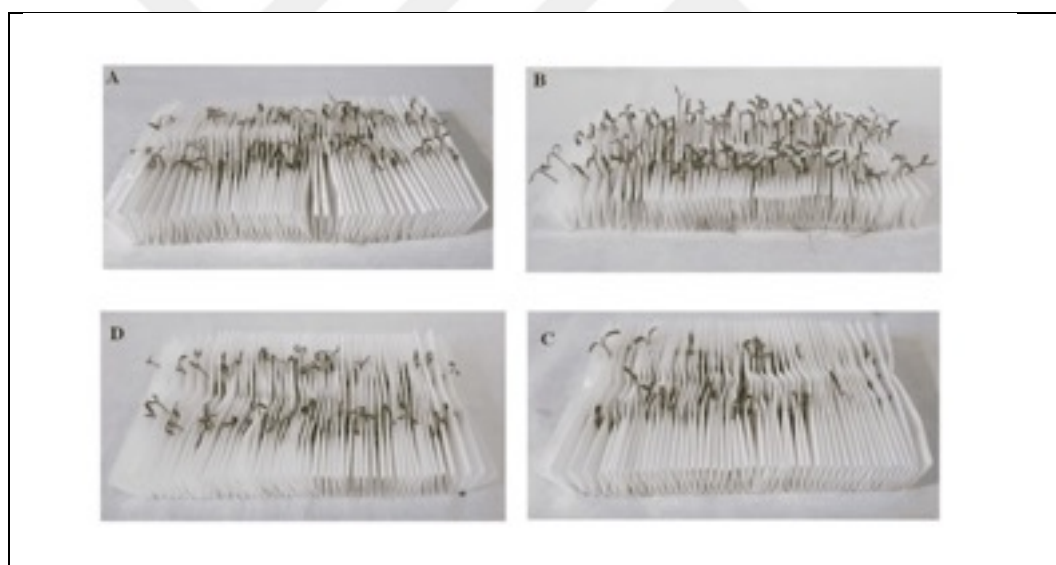


Figure 4.4. Comparison of control with hydro-priming effect on germination of the SG188 genotype of sugar beet in 10 days of post-priming. Figure A as control, Figure B-C-D shows hydro-priming 3, 6, and 9 hours respectively.

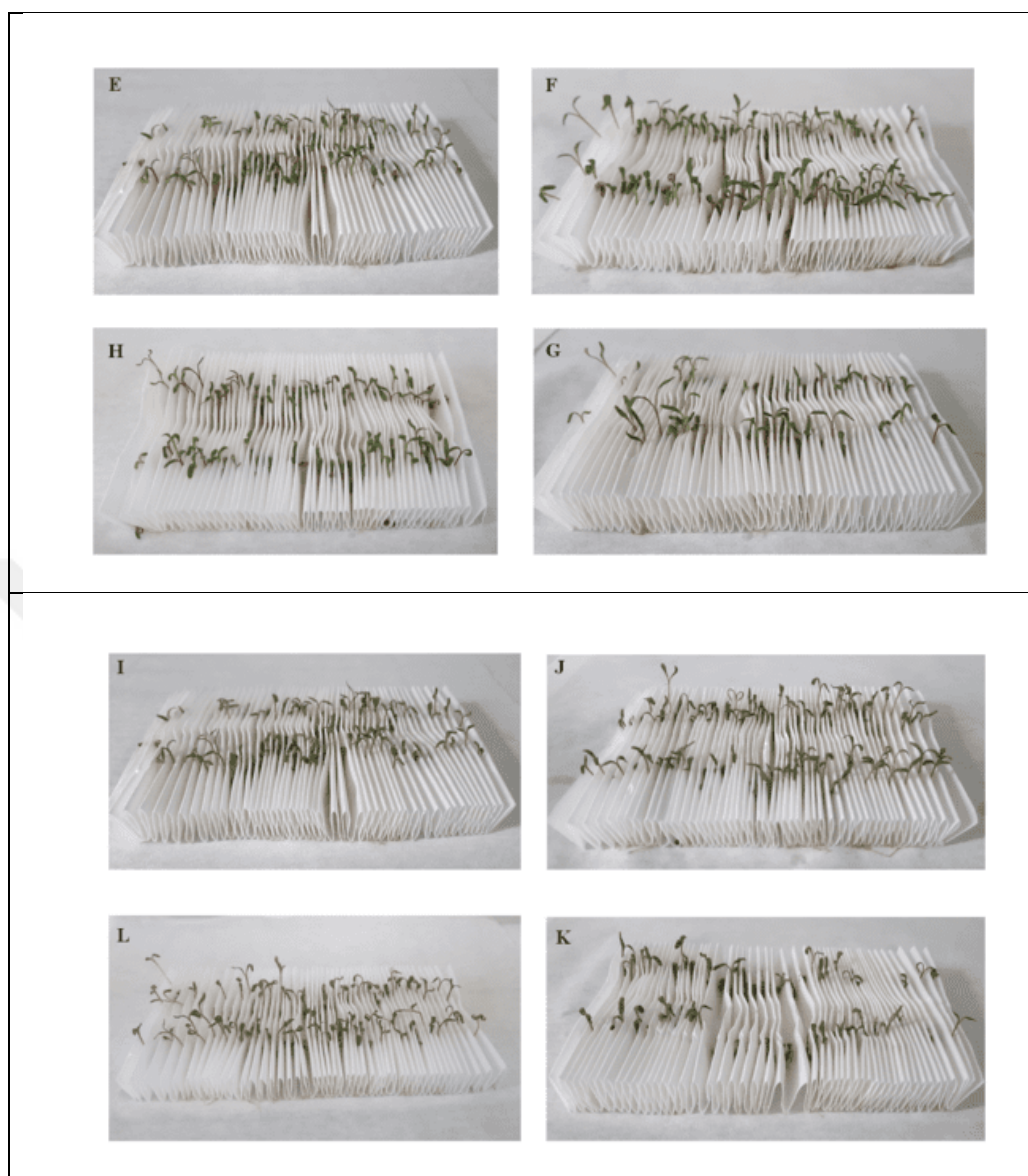


Figure 4.5. Comparison control sample with nano-priming in different concentration of titanium dioxide nanoparticles in different priming time (3hrs, 6 hrs, and 9 hrs) for 10 days post-priming during 10 days of growth. Figures A, E, I show control treatment. Germination of seeds after nano-priming is shown in Figures B, C, D with 5 ppm TiO₂ NPs, Figures F, G, H with 10 ppm TiO₂ NPs, Figures J, K, L with 15 ppm TiO₂ NPs for 3, 6 and 9 hrs respectively.

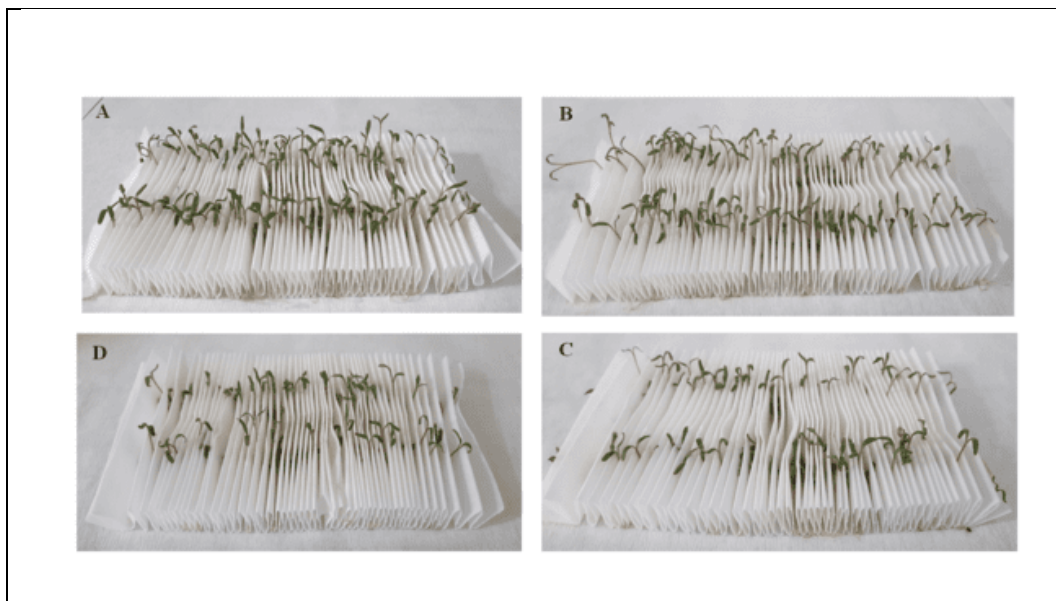


Figure 4.6. Comparison of control with hydro-priming in different priming time (3hrs, 6 hrs, 9 hrs) effect on germination of the SG188 genotype of sugar beet for 45 days of post-priming. Figures B-C-D show hydro-priming for 3, 6 and 9 hours, respectively.

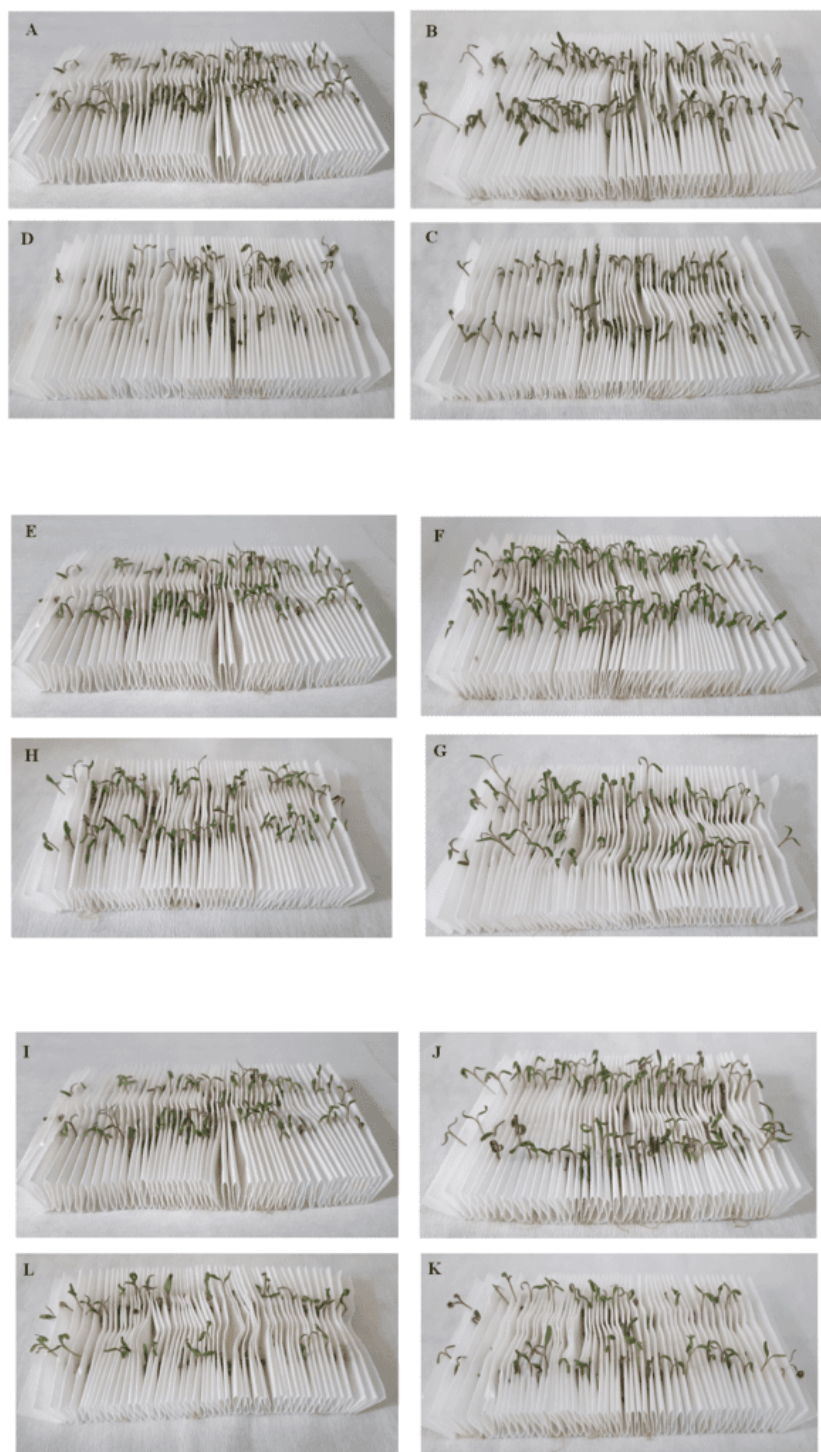


Figure 4.7. Comparison control sample with nano-priming in different concentration of titanium dioxide nanoparticles in different priming time (3hrs,6 hrs, and 9hrs) for 45 days post-priming during 10 days of growth. Figures A, E, I show control treatment. Figures B, C, D show nano-priming with 5 ppm TiO_2 NPs, Figures F, G, H with 10 ppm TiO_2 NPs, Figures J, K, L with 15 ppm TiO_2 NPs for 3, 6 and 9 hours, respectively.

4.1.3 The Effects of Priming on Germination Rate

The cumulative distributions of normal germination rates from the first counting days of germination performed following the control, hydro-priming, and nano-priming treatments in seeds of sugar beet SG188 are given in Figure 4.8.

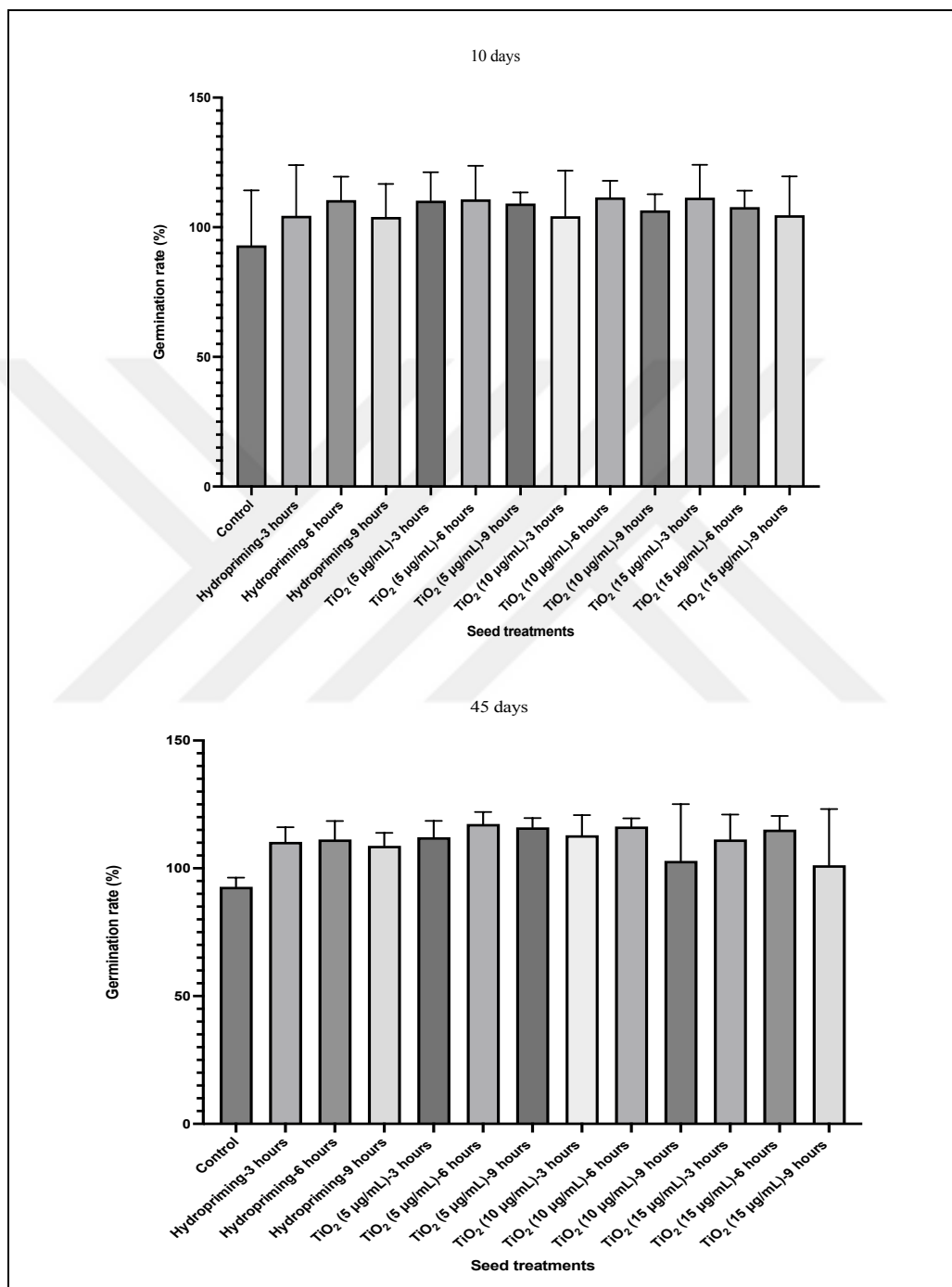


Figure 4.8. The changes in germination rates among control, priming with different concentrations of TiO₂ NPs (5 ppm, 10 ppm, and 15 ppm) as nano-priming, and hydro-priming treatments in seeds of SG188 sugar beet.

Germination rates of the seedlings have been measured by using the given formula in the method by changing in germination numbers by time. According to the results obtained from the treatments, on the second day after cultivating, which was the first count day, germination was observed, and the nano-priming and hydro-priming treatments were not significant. During the germination, it was determined that the increases in the germination rates of all treatment groups except the control group were close to each other. On the final count day, the beneficial effects of all treatments became more evident. While the treated with primed seeds exhibited a higher germination rate than the control group.

When the results obtained from the hydro-priming and nano-priming with TiO_2 NPs solutions with different concentrations (5ppm, 10 ppm, 15 ppm) treatment groups were considered together, it was observed that while the germination rate of the control group was 0.0% on the first count day, the germination rates of all treatment groups varied between 99% germination in post-priming 10 days compare with control (93.03 ± 21.16) as well as observed in post-priming 45 days showed same 99% germination in all primed seeds.

The results showed that both results for 10 days and 45 days control have the relatively lowest germination rates as 93.03 ± 21.16 and 92.77 ± 3.595 . Figure 4.8 represents the changes in the germination rates. Although the differences between the rates are insignificant statistically, it can be said that all priming processes are relatively effective on the rates.

4.1.4 The Effect on Water Content for Seed Priming

According to Figure 4.9, seeds with higher water content during priming exhibited shorter than average germination periods. Among all treatments, TiO_2 NPs with 10ppm concentration that was primed in solution at 3 hours had the highest water content in post-priming 10 days (27.25 ± 8.850) and after four days of cultivation had 95% germination.

Seeds primed with TiO_2 NPs at a concentration of 15 ppm for 6 hours exhibited the shortest germination period during the 45 days post-priming when the water content during priming was 17.24 ± 7.830 .

Both primed seeds in 10 days and 45 days had been treated at the same water content during priming. In conclusion, the ideal water content for sugar beet seeds during priming ranged from 15% to 25%, and the precise water content should be determined beforehand for the seed to be primed. It is probably that crack of root

extrusion or seeds appears when water content be more than these range during priming time.

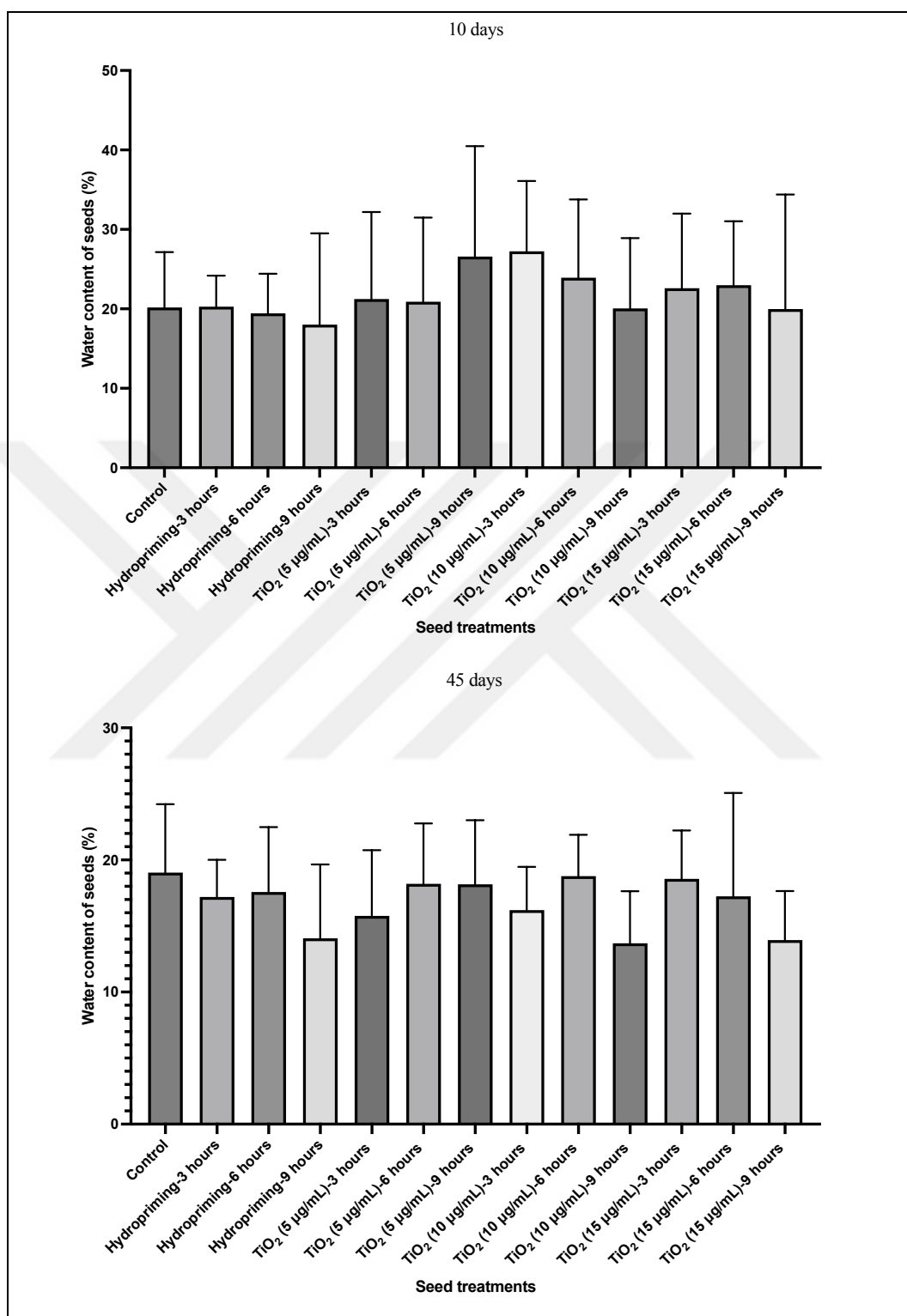


Figure 4.9. Comparison water content of sugar beet seeds for post-priming 10 days and 45 days.

4.1.5 The Effect on Root and Hypocotyl Length

Hydro-priming and nano-priming affected significantly ($<.001$) root and hypocotyl length. These graphs (Figure 4.10) showed that all treatments followed a role model by increasing priming time. In all treatments primed in solutions for about 9 hours a decrease in root length compared to the control group in post-priming both 10 days and 45 days.

The findings indicated that the highest average root length of 6.404 ± 0.8634 , 6.544 ± 1.670 , 2.685 ± 0.5221 cm belonged to nano-primed seeds that were in TiO_2 NPs (5 ppm, 10 ppm, 15 ppm) for 3 hours in post-priming 10 days, respectively). Whereas for unprimed seeds, the observed mean root length was 4.818 ± 0.7731 cm (Figure 4.10).

According to Figure 4.11 in post-priming 45 days represented those seeds were soaked with water solution for 3 hours had a higher mean root length of 6.265 ± 1.449 compared to nano-primed seeds and the control group (5.438 ± 1.160 cm).

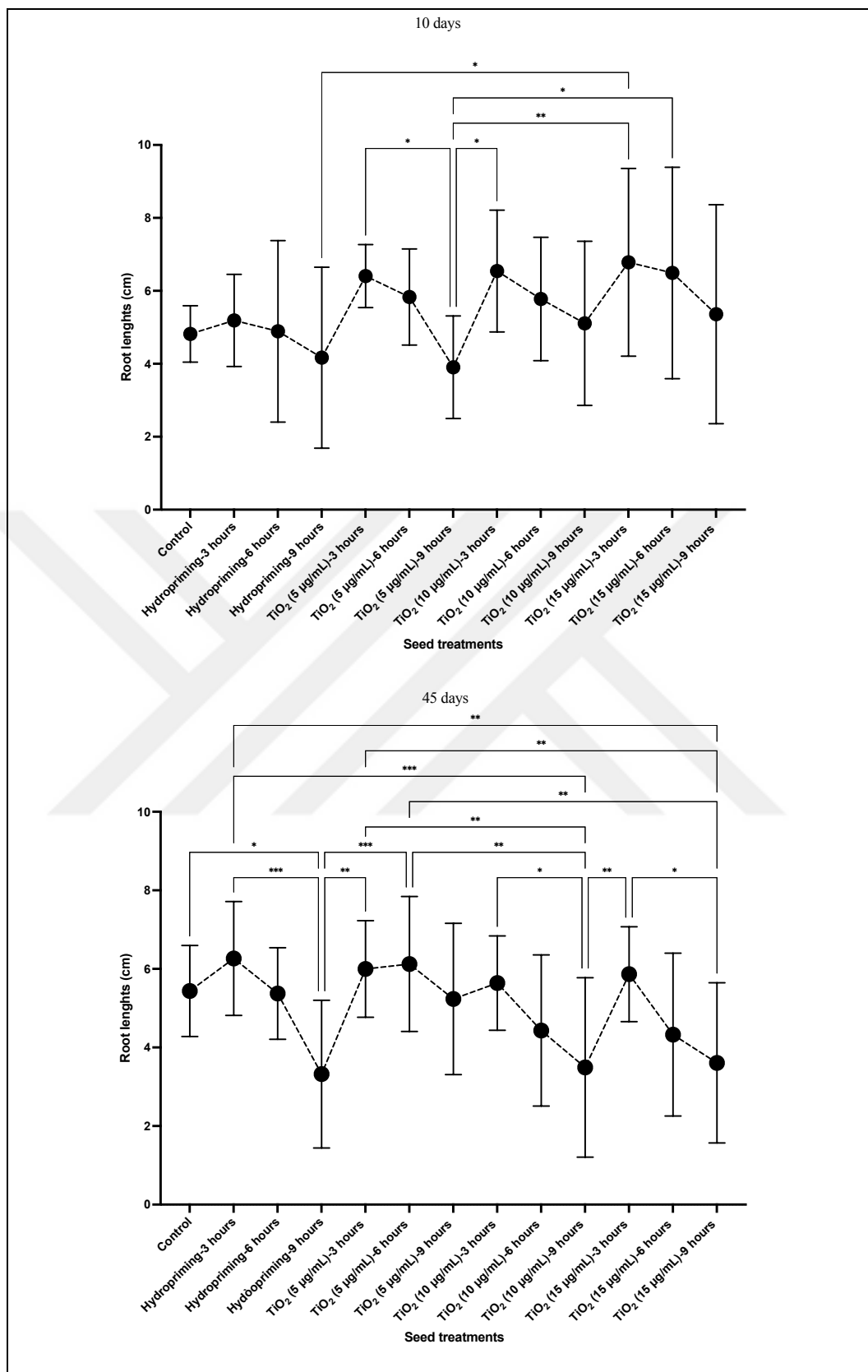


Figure 4.10. The effect of hydro-priming and nano-priming on SG188 sugar beet seeds in post-priming 10 days and 45 days. P value (<.001) represent **

The ANOVA analysis results also showed no significant differences in hypocotyl length among the treatment groups. Most treatments are close to control measurement. However, it was noticed that the seeds primed with TiO₂ NPs with a 15ppm concentration for 3 hours (2.685 ± 0.5221 cm), desirable result belonged to it. There was no relative change in post-priming 10 days.

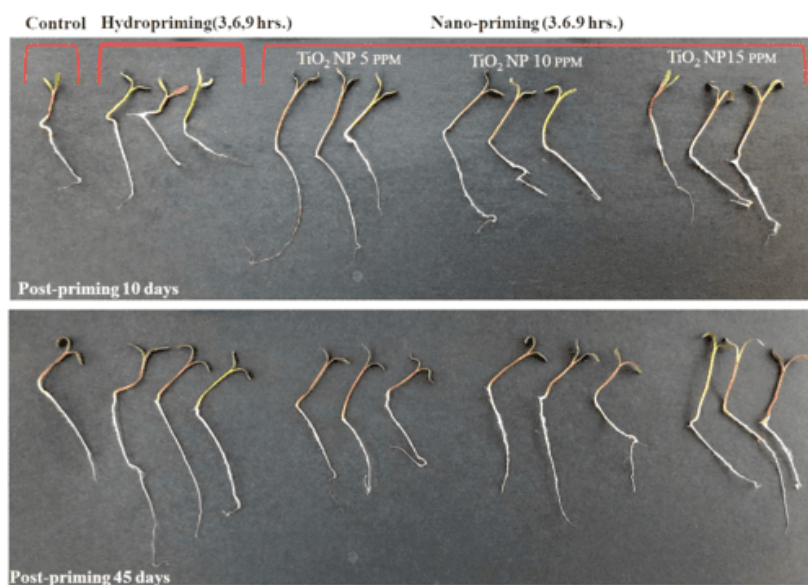


Figure 4.11. Morpho-physiological analysis during root development in control and primed seedling of SG188 sugar beet for 10 days of growth in post-priming 10, and 45 days.

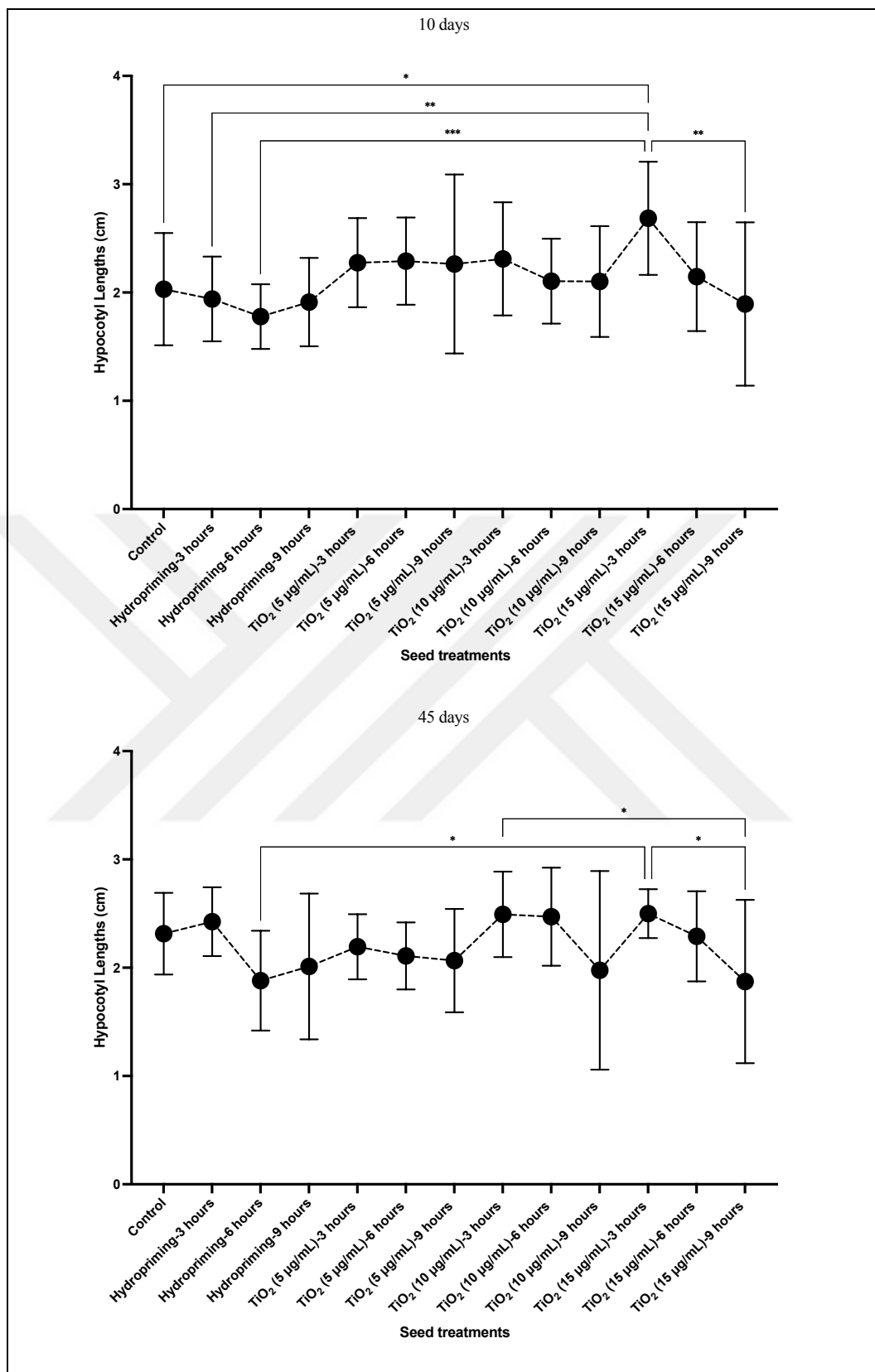


Figure 4.12. The effect of hydro-priming and nano-priming on SG188 sugar beet seeds in post-priming 45 days and 45 days. P value (<.001) represent ***

As shown in graph (Figure 4.12) about post-priming 45 days, hypocotyls seem to follow role models declining development by increasing primed time in all nanoparticle solutions in different concentrations of TiO₂ (5,10,15 ppm).

4.2 Physiological Assessment of Treated Seeds

4.2.1 α -amylase Activity

As shown in the graph (Figure 4.13) post-priming 10 days, the highest value of alpha-amylase was obtained in nano-priming seeds TiO₂ (10 ppm) for 3 hours (6.617 ± 0.2562 U/true seed) compared to primed seeds for 3,6 and 9 hours in water solutions by 2.118 ± 0.7480 , 5.158 ± 1.7423 , 5.79 ± 0.2237 U/true seed respectively, even it seems that alpha-amylase activity in TiO₂ (10 ppm) for 3 hours is double of hydro-priming for 3 hours. However, seeds primed with nanoparticle TiO₂ with a 10 ppm concentration gradually decreased by increasing times for priming. Moreover, the lowest was found in the seeds primed in water solution for 3 hours (2.118 ± 0.7480 U/true seed). There is significant P value (< 0.008).

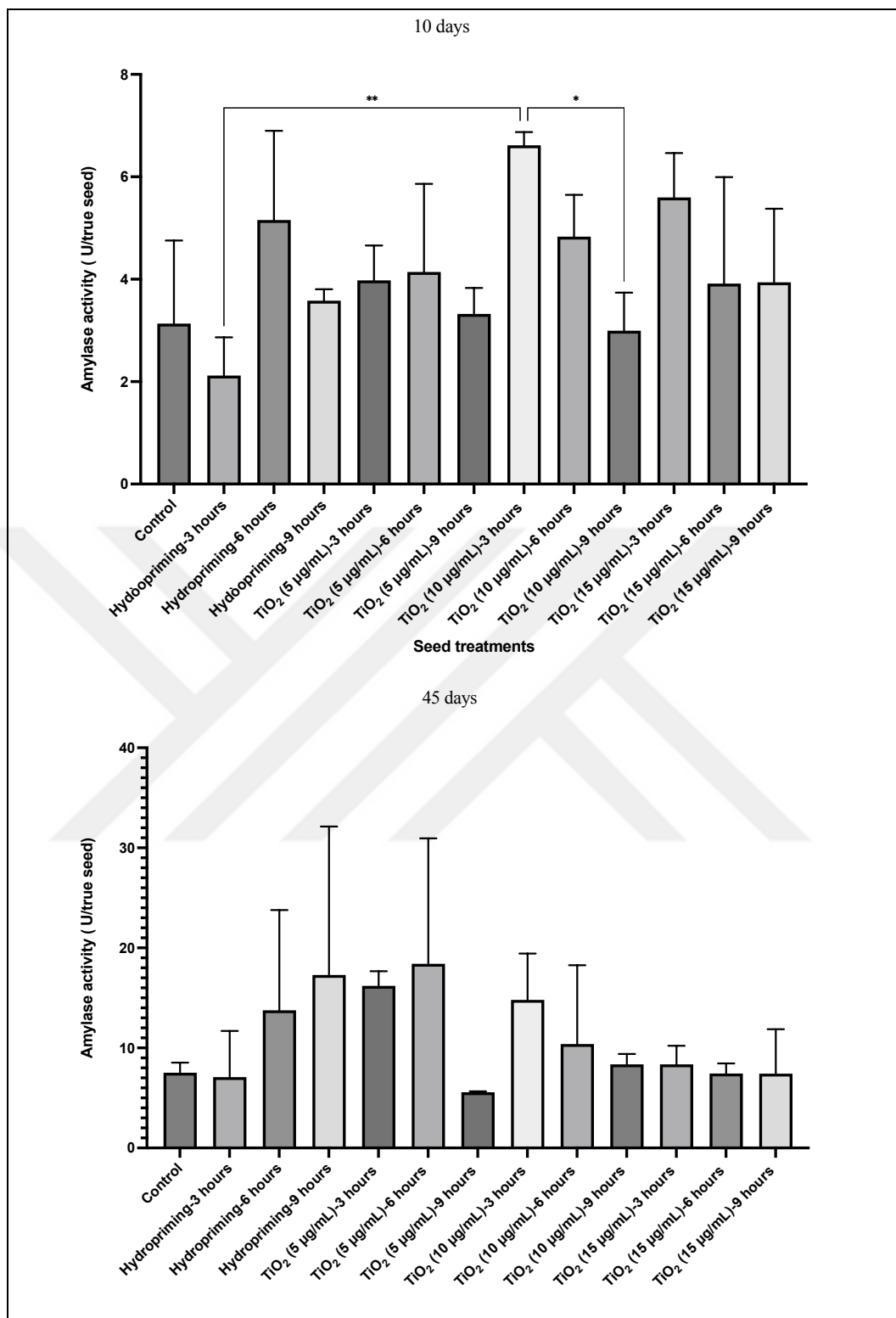


Figure 4.13. Comparison of alpha-amylase activities in seeds primed as hydro-priming and nano-priming with control in post-priming 10 and 45 days. A unit of amylase activity was defined as a 10% decrease in absorbance at 620 nm min⁻¹. P value of 10 days post priming (<.008) represent **

In the comparison of alpha-amylase activities between post-priming treatments, the highest alpha-amylase activities were seen in the seeds that were

cultivated after 45 days of priming. For nano-priming treatments primed seeds for TiO_2 5ppm, 6 hours the highest alpha-amylase activity with 18.41 ± 12.53 U/true seed. Through hydro-priming treatments seeds that are treated with distilled water for 9 hours showed the highest alpha-amylase activity (17.28 ± 14.84 U/true seed). Figure 4.13. as graph showed that by increased priming time with the concentration of TiO_2 NPs, 5 ppm had the lowest alpha-amylase activity among these treated seeds (5.577 ± 0.08796 U/true seed) even lower than control group (7.530 ± 1.007 U/true seed).

4.2.2 Chlorophyll Content

The statistical analysis based on ANOVA was conducted to examine the interactive effect of the priming technique on chlorophyll (Chl a) content, with significance value at $P < 0.05$. In post-priming 10 days, the impact of priming on Chl a was not found to be statistically substantial. The influence of control was like other treatments. All the employed this method statistically showed similar effects on Chl a. In contrast, post-priming 45 days was shown in primed seeds condition that the applied this technique had statistically significant on $P < 0.003$ (ANOVA) could increase Chl a content in comparison with the control by increasing priming-time especially as represented in figure 4.14 the highest Chl a belonged to seeds primed with TiO_2 NPs 10 ppm solution for 9 hours (46.02 ± 1.286 $\mu\text{g/mL}$), while the lowest Chl a content was in TiO_2 NPs 15 ppm treated for 3 hours (29.47 ± 1.649 $\mu\text{g/mL}$).

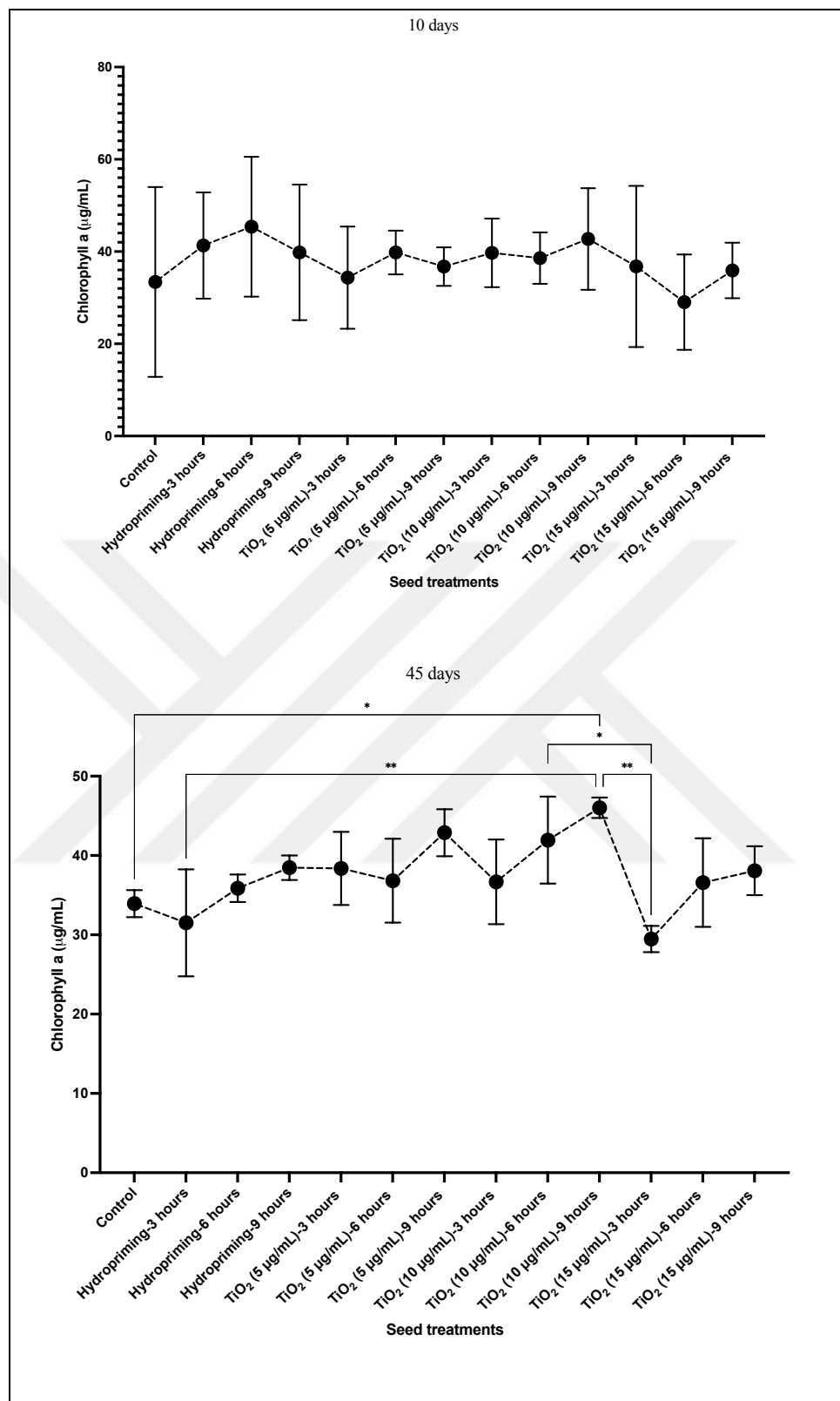


Figure 4.14. Effects priming on chlorophyll (a) content of seedlings which were primed in post-priming 10 and 45 days with water as hydro-priming and titanium dioxide nanoparticles (5,10,15 ppm concentrations).

Based on the results, it appeared that the priming effects on Chl b were distinct, but this was not statistically confirmed ($P < 0.05$) as shown in Figure 4.15.

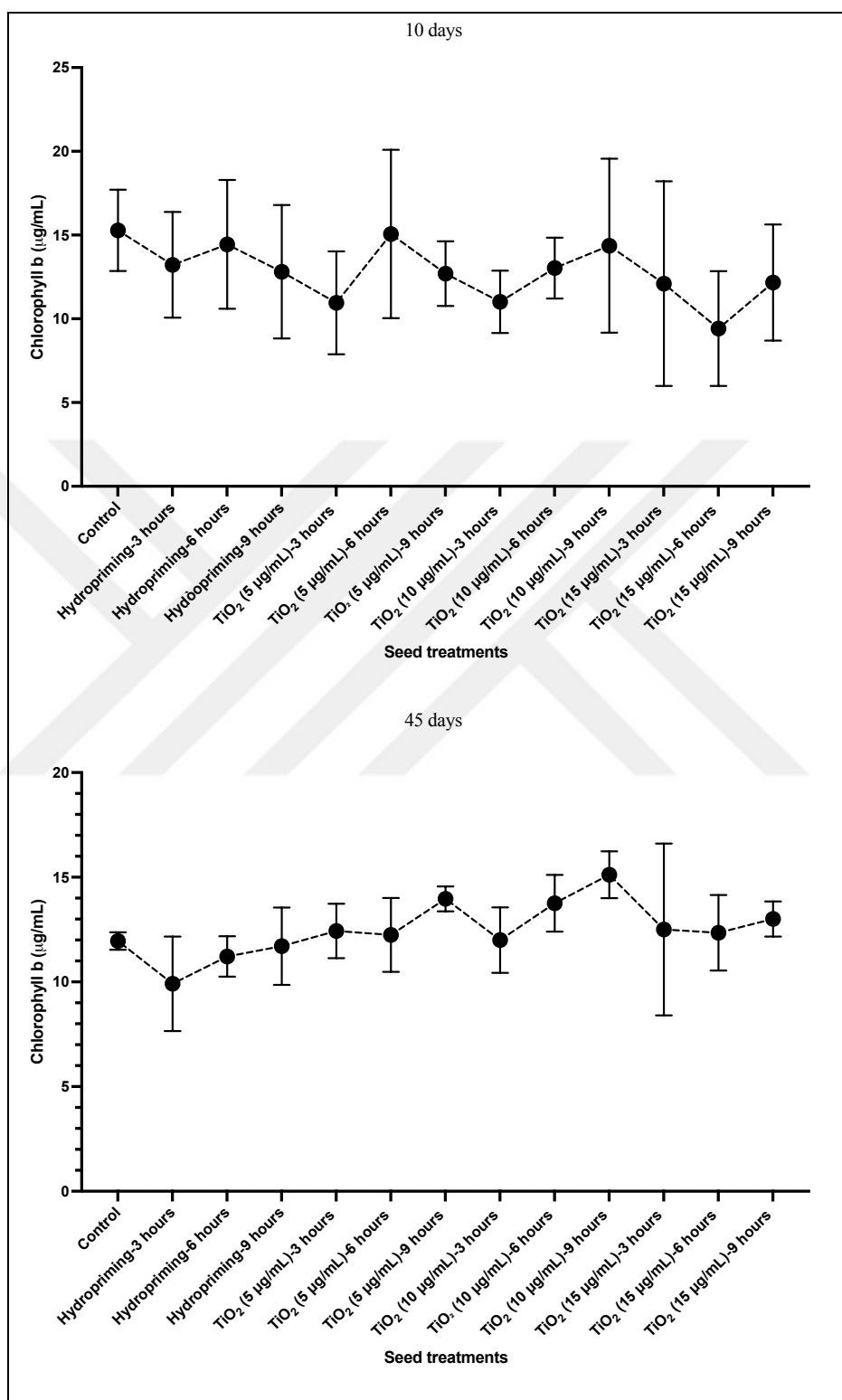
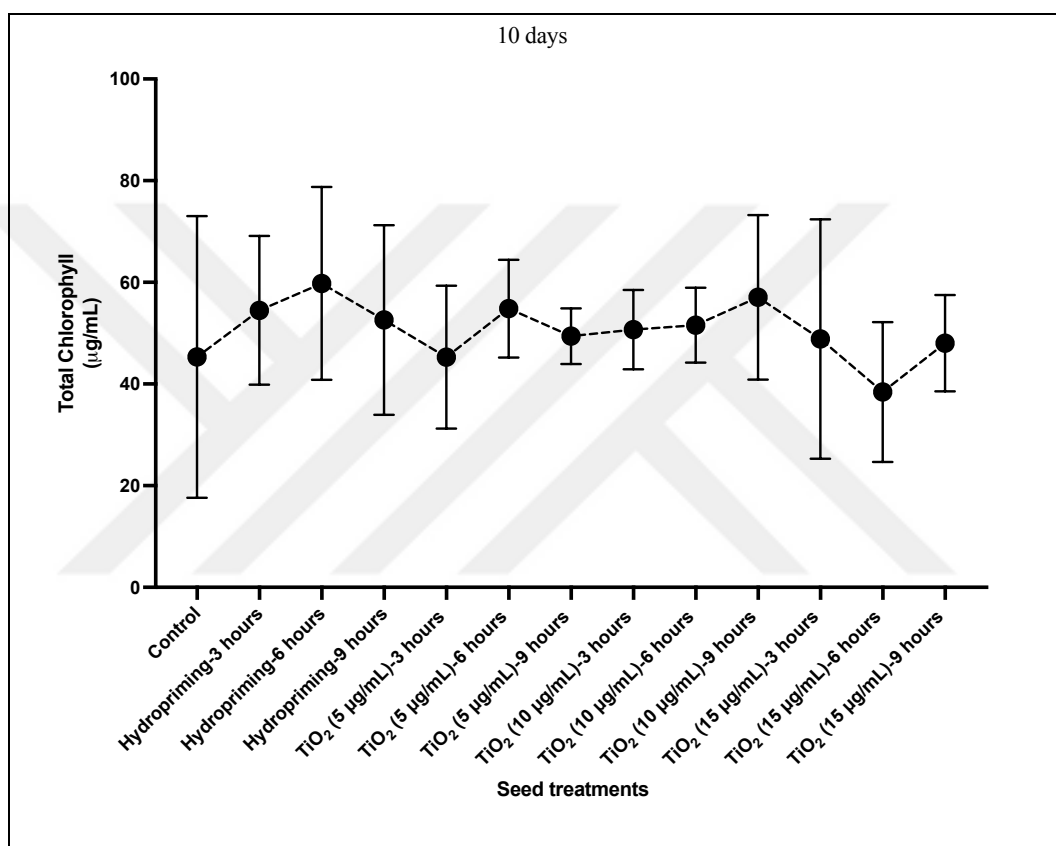


Figure 4.15. Effects priming on chlorophyll (b) content of seedlings which were primed in post-priming 10 and 45 days with water as hydro-priming and titanium dioxide nanoparticles (5,10,15 ppm concentrations).

The total amount of Chl a+b (Chl T) was significantly altered by the applying of the priming technique post-priming in 45 days with statistically significant on $P < 0.006$ (ANOVA). It was noticed in the graph (Figure 4.16) that they were gradually boosted by increasing priming time, the highest chlorophyll total belonged to seedlings that primed seeds with titanium dioxide at 10 ppm concentrations for 9 hours ($61.10 \pm 1.811 \mu\text{g/mL}$) in comparison to other treatments and control groups ($45.85 \pm 1.747 \mu\text{g/mL}$) in 45 days as post-priming.



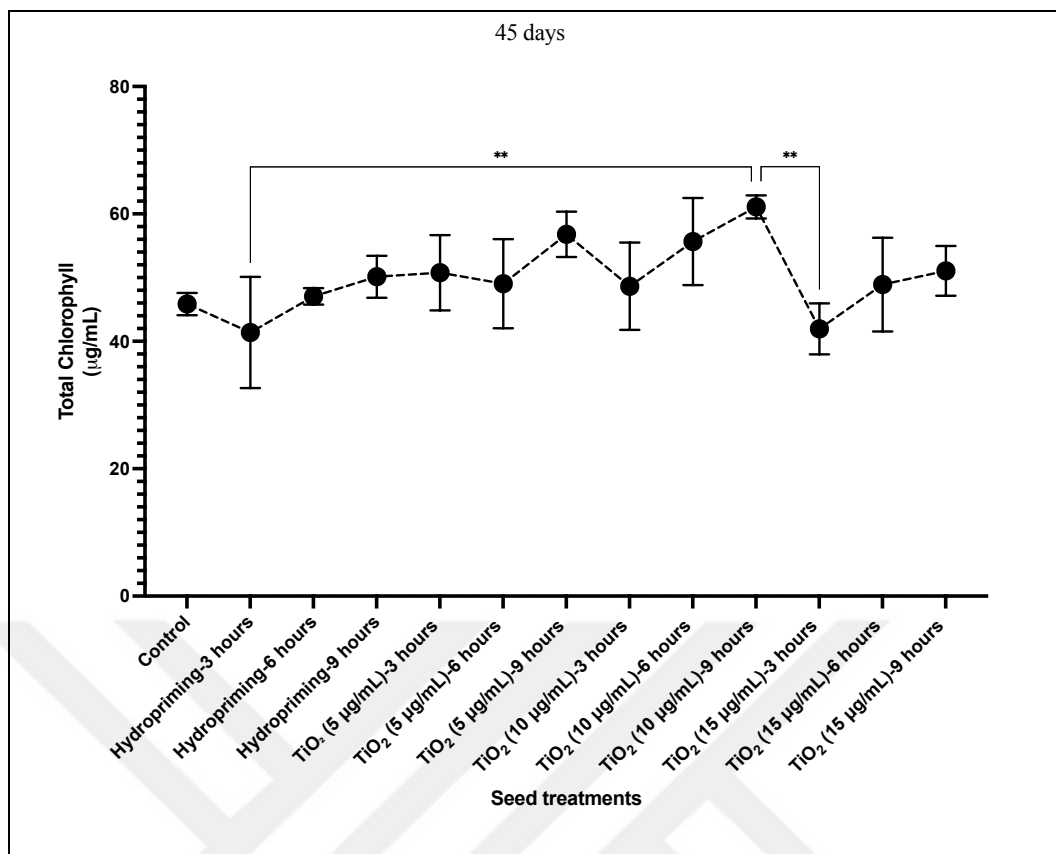


Figure 4.16. Effects priming on Chlorophyll Total (Chl-T) content of seedlings which were primed in post-priming 10 and 45 days with water as hydro-priming and titanium dioxide nanoparticles (5,10,15 ppm concentrations).

4.2.3 MDA Content

The effects of hydro-priming and nano-priming on the MDA content of the sugar beet plants were not found to be statistically significant at $P < 0.05$ (ANOVA) for both the 10- and 45-days post-priming periods. However, as shown in the graph (Figure 4.17), the MDA content in seedlings for post-priming 45 days was higher than in seedlings that were grown after 10 days of priming. All hydro-priming treatments and nano-priming followed the raising model with increasing priming time except seedlings primed seeds with titanium dioxide at 15 ppm concentrations for 9 hours (0.6366 ± 0.4796 nmol/mL). While post priming after 45 days followed this model in seedlings treated with TiO₂ NP 5, 10 ppm concentrations in 3, 6 and 9 hours priming time. It is related to TiO₂ NP 5 ppm, 0.7097 ± 0.3414 , 0.8387 ± 0.2812 , 1.032 ± 0.2326 nmol/mL and TiO₂ NP 10 ppm, 0.8172 ± 0.1971 , 0.9032 ± 0.2326 , 1.075 ± 0.4755 nmol/mL respectively.

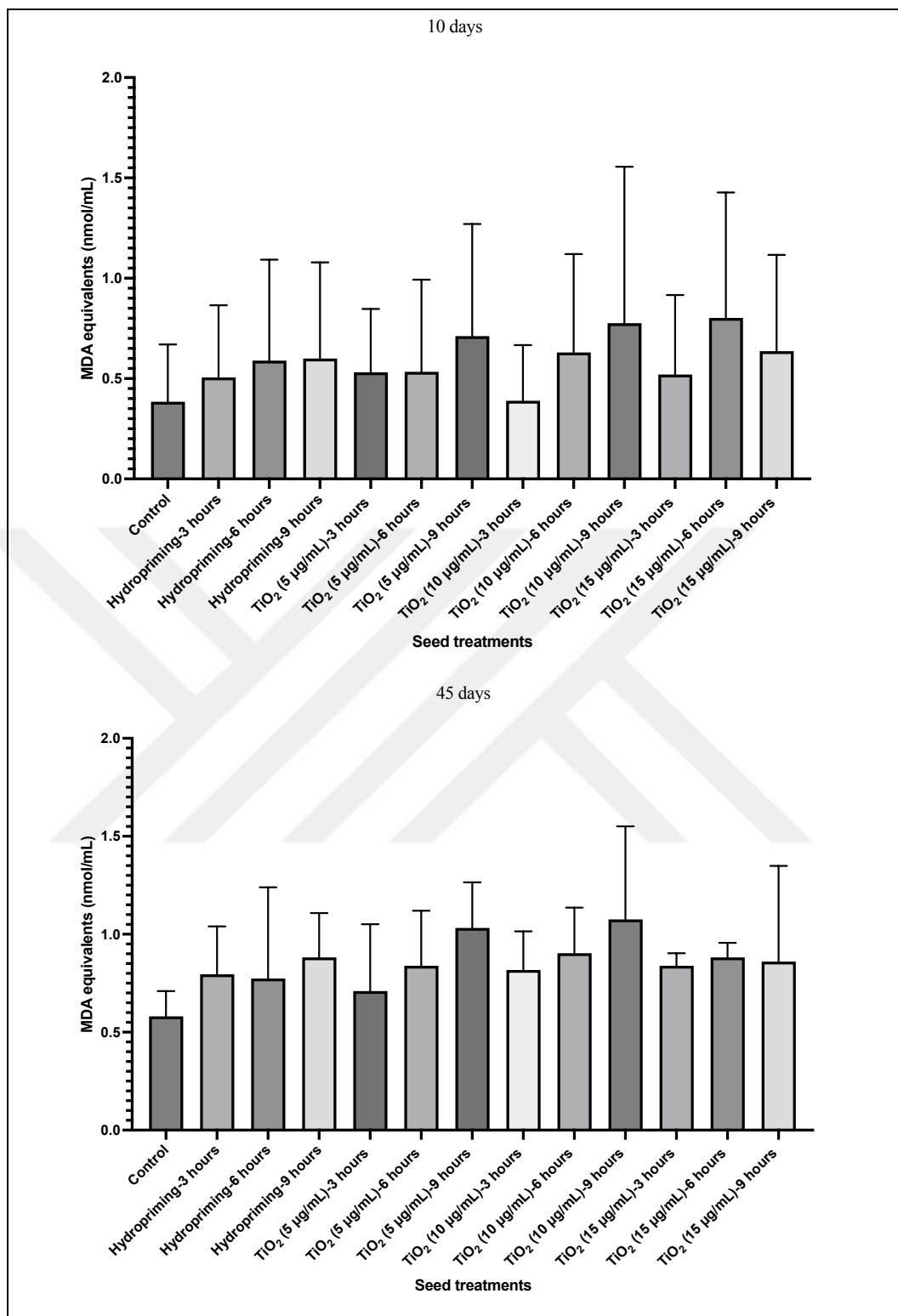


Figure 4.17. Impacts of the priming on malondialdehyde (MDA) content of the sugar beet plants.

All results of the studies carried out during the thesis are presented respectively in the tables below.

Table 4. 1. Effects of different priming solutions, priming time on germination indices and physiological characteristics of *Beta vulgaris* L. genotype in 10 days post-priming seed maintenance.

	GP%	GR%	SVI	Water content (%)	RL (cm)	HL (cm)
Control	93.33± 21.16	93.03± 21.16	639.22± 168.97	20.18± 6.968	4.818± 0.7731	2.031± 0.5183
Hydropriming 3 hours	97.00± 3.606	104.4± 19.56	691.42± 130.71	20.27± 3.911	5.188± 1.262	1.940± 0.3910
Hydropriming 6 hours	95.67± 2.082	110.5± 9.058	638.02± 240.14	19.44± 4.993	4.891± 2.488	1.778± 0.2988
Hydropriming 9 hours	96.33± 4.041	104.0± 12.70	585.69± 243.45	18.02± 11.49	4.168± 2.481	1.912± 0.4084
TiO₂ (5 µg/mL) 3 hours	98.00± 2.000	110.2± 10.98	850.54± 95.33	21.24± 10.95	6.404± 0.8634	2.275± 0.4116
TiO₂ (5 µg/mL) 6 hours	97.00± 0.000	110.7± 12.95	787.74± 133.68	20.90± 10.59	5.831± 1.318	2.290± 0.4028
TiO₂ (5 µg/mL) 9 hours	95.33± 1.155	109.1± 4.325	587.90± 155.70	26.57± 13.88	3.904± 1.407	2.263± 0.8260
TiO₂ (10 µg/mL) 3 hours	96.00± 1.732	104.2± 17.62	850.08± 168.67	27.25± 8.850	6.544± 1.670	2.311± 0.5221
TiO₂ (10 µg/mL) 6 hours	97.00 ± 1.000	111.5± 6.421	764.65± 168.55	23.91± 9.871	5.778± 1.691	2.105± 0.3915
TiO₂ (10 µg/mL) 9 hours	95.00± 2.000	106.5± 6.213	816.34± 280.09	20.05± 8.858	6.491± 2.898	2.102± 0.5111
TiO₂ (15 µg/mL) 3 hours	99.00± 1.000	111.4± 12.67	937.23± 260.28	22.60± 9.381	6.782± 2.575	2.685± 0.5221
TiO₂ (15 µg/mL) 6 hours	96.33± 3.215	107.8± 6.367	832.10± 284.69	22.99± 8.042	6.491± 2.898	2.147± 0.5029
TiO₂ (15 µg/mL) 9 hours	95.00± 1.732	104.6± 15.00	689.04± 294.32	19.98± 14.40	5.360± 3.002	1.893± 0.7543

Table 4.2. Effects of different priming solutions, priming time on germination indices and physiological characteristics of *Beta vulgaris* L. genotype in 45 days post-priming seed maintenance.

	GP%	GR	SVI	Water content (%)	RL (cm)	HL (cm)
Control	93.67± 5.774	92.77± 3.595	726.13± 122.69	19.03± 5.173	5.438± 1.160	2.314± 0.3765
Hydropriming 3 hours	94.33± 7.371	110.3± 5.718	819.73± 153.89	17.19± 2.816	6.265± 1.449	2.425± 0.3177
Hydropriming 6 hours	97.00± 2.000	111.3± 7.186	703.64± 122.48	17.58± 4.910	5.374± 1.166	1.880± 0.4609
Hydropriming 9 hours	94.00± 1.000	108.8± 5.048	501.30± 187.67	14.07± 5.586	3.321± 1.879	2.012± 0.6724
TiO₂ (5 µg/mL) 3 hours	95.67± 1.528	112.2± 6.361	783.73± 121.80	15.76± 4.973	5.999± 1.230	2.193± 0.3012
TiO₂ (5 µg/mL) 6 hours	97.67± 2.517	117.3± 4.706	804.21± 171.75	18.19± 4.575	6.125± 1.718	2.109± 0.3092
TiO₂ (5 µg/mL) 9 hours	97.33± 0.5774	116.0± 3.651	710.51± 193.06	18.15± 4.860	5.234± 1.925	2.066± 0.4764
TiO₂ (10 µg/mL) 3 hours	98.33± 1.155	112.9± 7.900	799.52± 124.76	16.20± 3.281	5.639± 1.202	2.492± 0.3950
TiO₂ (10 µg/mL) 6 hours	97.00± 1.000	116.3± 3.235	669.59± 191.86	18.77± 3.127	4.432± 1.924	2.471± 0.4531
TiO₂ (10 µg/mL) 9 hours	90.67± 7.767	102.9± 22.23	495.78± 227.07	13.69± 3.948	3.492± 2.283	1.976± 0.9168
TiO₂ (15 µg/mL) 3 hours	97.33± 1.528	111.3± 9.768	814.17± 120.19	18.56± 3.666	5.865± 1.207	2.500± 0.2255
TiO₂ (15 µg/mL) 6 hours	98.33± 1.528	115.1±5.346	650.75± 208.05	17.24± 7.830	4.328± 2.072	2.290± 0.4160
TiO₂ (15 µg/mL) 9 hours	91.33± 6.429	101.2± 21.98	500.40± 201.57	13.93± 3.720	3.607± 2.038	1.872± 0.7541

Table 4.3. Effects of different priming solutions, priming time on biochemical characteristics of *Beta vulgaris* L. genotype in 10 days post-priming seed maintenance.

	Chl_a (µg/mL)	Chl_b (µg/mL)	Chl_T (µg/mL)	α-amylase (U/true seed)	MDA (nmol/mL)
Control	33.41±20.57	15.28±2.427	45.32±27.72	3.132±1.625	0.3849±0.2849
Hydropriming 3 hours	41.31±11.52	13.22±3.156	54.49±14.63	2.118±0.7480	0.5054±0.3597
Hydropriming 6 hours	45.39±15.14	14.44±3.844	59.79±18.97	5.158±1.742	0.5892±0.5033
Hydropriming 9 hours	39.82±14.69	12.81±3.985	52.60±18.66	3.579±0.2237	0.6000±0.4792
TiO₂ (5 µg/mL) 3 hours	34.35±11.07	10.96±3.073	45.27±14.05	3.978±0.6820	0.5312±0.3158
TiO₂ (5 µg/mL) 6 hours	39.80±4.748	15.06±5.027	54.83±9.613	4.143±1.718	0.5333±0.4599
TiO₂ (5 µg/mL) 9 hours	36.75±4.167	12.69±1.926	49.41±5.475	3.321±0.5077	0.71118±0.5587
TiO₂ (10 µg/mL) 3 hours	39.72±7.435	11.01±1.862	50.70±7.791	6.617±0.25623	0.3892±0.2775
TiO₂ (10 µg/mL) 6 hours	38.59±5.568	13.03±1.817	51.58±7.379	4.831±0.8165	0.6301±0.4903
TiO₂ (10 µg/mL) 9 hours	42.73±11.01	14.36±5.195	57.05±16.18	2.995±0.7438	0.7764±0.7793
TiO₂ (15 µg/mL) 3 hours	36.78±17.47	12.10±6.109	48.85±23.56	5.597±0.8686	0.5204±0.3957
TiO₂ (15 µg/mL) 6 hours	29.03±10.33	9.418±3.425	38.42±13.75	3.916±2.079	0.8022±0.6254
TiO₂ (15 µg/mL) 9 hours	35.90±6.023	12.17±3.468	48.03±9.485	3.941±1.435	0.6366±0.4796

Table 4.4. Effects of different priming solutions, priming time on biochemical characteristics of *Beta vulgaris* L. genotype in 45 days post-priming seed maintenance.

	Chl_a (µg/mL)	Chl_b (µg/mL)	Chl_T (µg/mL)	α-amylase (U/true seed)	MDA (nmol/mL)
Control	33.93±1.704	11.95±0.4180	45.85±1.747	7.530±1.007	0.5806±0.1291
Hydropriming 3 hours	31.51±6.746	9.910±2.259	41.39±8.722	7.086±4.609	0.7957±0.2443
Hydropriming 6 hours	35.87±1.738	11.22±0.9624	47.06±1.304	13.75±10.02	0.7742±0.4652
Hydropriming 9 hours	38.46±1.551	11.71±1.846	50.13±3.282	17.28±14.84	0.8817±0.2266
TiO₂ (5 µg/mL) 3 hours	38.37±4.610	12.43±1.300	50.77±5.905	16.20±1.478	0.7097±0.3414
TiO₂ (5 µg/mL) 6 hours	36.82±5.282	12.24±1.762	49.03±7.001	18.41±12.53	0.8387±0.2812
TiO₂ (5 µg/mL) 9 hours	42.87±2.967	13.97±0.5928	56.80±3.559	5.577±0.08796	1.032±0.2326
TiO₂ (10 µg/mL) 3 hours	36.68±5.338	12.00±1.560	48.65±6.848	14.81±4.620	0.8172±0.1971
TiO₂ (10 µg/mL) 6 hours	41.94±5.487	13.76±1.357	55.67±6.829	10.38±7.884	0.9032±0.2326
TiO₂ (10 µg/mL) 9 hours	46.02±1.286	15.12±1.120	61.10±1.811	8.353±1.044	1.075±0.4755
TiO₂ (15 µg/mL) 3 hours	29.47±1.649	12.51±4.103	41.95±4.000	8.360±1.863	0.8387±0.06450
TiO₂ (15 µg/mL) 6 hours	36.59±5.584	12.35±1.807	48.90±7.3575	7.439±1.008	0.8817±0.07448
TiO₂ (15 µg/mL) 9 hours	38.08±3.075	13.01±0.8403	51.06±3.904	7.425±4.439	0.8602±0.4885

5. DISCUSSION

Seed priming is the most appropriate and simple technique among various types of techniques that are applied in agriculture to improve growth and yield of production. This technique synchronizes germination, improving emergence and crop establishment (Devi et al., 2023). This process utilizes an osmotic solution to intake water for a specific duration, followed by drying to restore their initial moisture content. Priming involves controlled hydration and subsequent drying of seeds before germination, aiming for accelerated germination (Tuğrul, 2022). The germination process in sugar beet is predominantly regulated by the pericarp, which acts as a physical and chemical barrier and reservoir of inhibitory substances. Previous studies have suggested that the operculum and basal pores are primary entry points for water and oxygen during germination (Santos & Pereira, 1989). Farmers have a high demand for high-quality seeds with exceptional germination performance, which necessitates the use of costly seeds and processing technologies that significantly contribute to the commercial value of sugar beet fruits. These treatment methods often positively impact sugar beet germination performance by optimizing the speed, capacity, and uniformity of germination and seedling establishment in the field (Ignatz et al., 2019).

In this research, the impacts of hydro-priming and nano-priming on sugar beet (*Beta vulgaris*) seeds were investigated to reveal the most suitable methods in terms of economy and science for the seed industry of sugar beet. The results obtained from all the treatments were evaluated based on the viability physiology, morphology, and biochemistry analysis parameters.

To observations in this finding suggest that the control group and primed seeds which were treated with distilled water and nanoparticle solutions with different concentrations of TiO_2 (5,10,15 ppm), priming times (3,6, and 9 hrs.), and post-priming (10- and 45-days storage seed primed) in terms of germination percentage and rate, there was no difference between them. The germination rate and seedling vigor of the primed seeds showed an increase compared to the control group, but these results are not statistically significant. The reaction to priming is influenced by diverse seed morphological factors, including cultivar type, initial quality, maturity level, and the specific priming method employed. Species with high variability of seed could achieve more advantages from this technique.

Moreover, priming demonstrates high effectiveness through seeds that have lower vigor (Chomontowski et al., 2020; Paparella et al., 2015; Salimi & Boelt, 2019).

On the side, it seems that the plant was not sensitive to nanoparticles, and also the concentrations used in this research. Titanium dioxide nanoparticle solutions, time of soaked seeds inside them, and even priming maintenance in 10- and 45-days post-priming as seed longevity did not have any adverse effects such as toxicity on germination of the plant. Some research suggests that the process of seed priming, particularly with low concentrations of TiO₂ nanoparticles, is more effective in enhancing the germination of wheat seeds (*Triticum aestivum* L.) and promoting seedling growth compared to bulk TiO₂ and untreated seeds (Faraji & Sepehri, 2019; Feizi et al., 2012). However, opposite to these observations, (Haghighi & Teixeira da Silva, 2014) showed that high concentrations of TiO₂ nanoparticles had the most positive impact on germination in Tomatoes (*Lycopersicon esculentum* Mill.), Onion (*Allium cepa* L.), and Radish (*Raphanus sativus* L.).

On the other side, it showed that the soaking time of seeds in a primed solution affects morphogenes of the plant. By increasing the time of soaking in hydro and nano solutions (9 hours as priming time), the hypocotyl and root length of seedlings decreased in sugar beet for 10 and 45 days after priming. Elamrani et al., (1992) emphasized that lipids in the embryo functioned as the primary respiratory substance for sugar beet during germination. They observed that starch was mainly hydrolyzed after the extrusion of the root, boosting the elongation of both the root and hypocotyl. The data obtained in the study are compatible with this information, and after both 10 days and 45 days of postpriming application, root lengths were relatively greater than the control for each application, and a limited decrease was observed as the priming period was prolonged (3, 6 and 9 hours). Although there is a decrease in hypocotyl length in the hydropriming application, it is not statistically significant. In other results, the changes are very close to each other.

Earlier research by Faraji & Sepehri, (2019) it was noted the impacts of TiO₂ nanoparticles (0, 500, 1000, and 2000 mg.L) on wheat seed germination and seedling growth under PEG-induced drought stress (0, -0.4, and -0.8 MPa) were similar to the investigation mentioned here. The results revealed that the germination percentage (GP) and germination rate (GR) increased by up to 23.72%

and 33.74%, respectively. Other studies (Feizi et al., 2012; Mahmoodzadeh & Aghili, 2014) have also observed enhanced seed germination and seedling growth in wheat due to TiO₂ NPs exposure. It is suggested that TiO₂NPs may induce oxidation-reduction reactions involving the superoxide ion radical during germination, scavenging free radicals in germinating seeds. Moreover, the oxygen produced in this process could enhance germination by aiding in respiration. Additionally, nanoparticles potentially create larger pores in the seed coat, facilitating water uptake within the seed embryo and ultimately accelerating the germination process (Faraji & Sepehri, 2019). The use of TiO₂NPs on maize seeds resulted in an increase in root-shoot length, as well as fresh and dry weights of seedlings, under both normal and salinity stress conditions, according to Shah et al., (2021b). The nano-priming with TiO₂ in this investigation demonstrated superior efficiency in enhancing seedling growth compared to hydro-priming. Enhanced relative water content, K⁺ concentration, and increased total proline and phenolic contents were observed following TiO₂NPs treatment, providing support for seedling growth. The findings (Tamindžić et al., 2023) showed that the metal-based of nano-priming treatment (Nanoplant Ultra) tested had no significant impact on the germination of the Dukat and Partner pea cultivars when compared to the control group, additionally, hydropriming resulted in a reduction in the germination in the Dukat cultivar. In other research (Pirasteh-Anosheh & Hamidi, 2013) showed that the utilization of urea priming with PEG (6000) priming at four levels: 0 (control), - 0.05, -0.10 and -0.15 bar, not only does not improve germination but actually decreases its rate. As the osmotic potential increases, the reduction in germination rate becomes more evident. Additionally, as the concentration of urea needs to be elevated, resulting in higher osmotic potential, there is a noticeable decline in germination rate. In Adhikari et al., (2022) The *Lactuca sativa* (cv. ‘Burpee Bibb’) seeds were treated with 0.05% potassium nitrate, 3 mM gibberellic acid, and distilled water. All the primed and non-primed seeds were subjected to 100 mM sodium chloride (NaCl) or 0 mM NaCl (control). No interaction effect was observed between different priming and salt treatments, except for the synchronization index as one of the germination parameters ($p < 0.05$). Hydro-primed seeds were better synchronized under salt stress. In contrast, other priming methods under the stress increased membrane permeability, leading to osmotic stress and tissue damage

Furthermore, Nanoparticles (NPs) have the potential to interrupt seed dormancy by increasing nutritional and water absorption, hence raising the seed vigor, and facilitating subsequent seedling growth. In a similar way, the priming of maize (*Zea mays* L.) seeds with TiO₂NPs (60 ppm) had a significant impact on germination rate and seedling vigor under salt stress, attributed to enhanced antioxidant enzyme activities (Shah et al., 2021a). In a recent study, it was found that SeNPs and ZnONPs altered the expression of ABA and gibberellin genes in germinating rapeseed under salinity stress conditions. This modulation led to increased germination rates in primed seeds compared to both unprimed and hydro-primed seeds (El-Badri et al., 2021).

Seed priming results in heightened activities of various enzymes involved in protein metabolism (proteases), carbohydrate metabolism (α and β amylases), and lipid metabolism (isocitrate lyase), facilitating the mobilization of stored reserves in seeds (Sisodia et al., 2018; Varier et al., 2010). Enzymes play a vital role in breaking down complex molecules to support the growth and development of the embryo. This process has a significant impact on the early and improved seedling growth emergence (Varier et al., 2010). In this study, ANOVA analysis showed a significant variable among seeds treated with water and various concentrations of nanoparticles. Especially, there was a statistically significant increase in alpha-amylase enzymes in seeds treated with nanoparticles compared to the control group (Figures 4.12). The enhance in alpha-amylase activity after priming is a phenomenon that has been observed in several species, including maize, marigold, milk thistle, muskmelon, oregano, rice, and sugar beet (Afzal et al., 2012; Basra et al., 2005; Farashah et al., 2011; FJ, 1993; Lee & Kim, 1999, 2000; Sathish & Sundareswaran, 2010; Sedghi et al., 2010; G. Singh et al., 1999; Szopińska & Politycka, 2016). Mukasa et al., (2003) reported a 1.9 to 11.5 times higher amylase activity in primed sugar beet seeds compared to control seeds, suggesting activation of metabolism not only in the embryo but also in the endosperm or perisperm. While in this research by increasing the time of seed priming in nano-priming conditions observed in both 10 and 45 days after primed, α -amylase enzyme had tended to decrease. This suggests that the storage period has not exerted a significant influence on the activity of the α -amylase enzyme. However, this enzyme activity was higher in hydro-primed seed to compare nano-treated and control groups. According to the result, it could be claimed that

indicating the adverse effects from TiO₂ NPs the increased duration of priming NPs (9 hours) exhibited toxicity in seeds of sugar beet. While Laware & Raskar, (2014) indicated that a low concentration of TiO₂ NPs improved the percentage of seed germination and growth rate of seedling with a simultaneous increase in the amylase and protease activities. Then, factors including concentration, form, shape, and duration of application from nanoparticles will affect the priming technique.

Khan et al., (2013) found that in recently harvested pea seeds, the activity of alpha- and beta-amylase enzymes was higher compared to seeds stored for longer periods and observed a decrease in enzyme activity as the storage duration prolonged, correlating with a decline in seed vigor. Similarly, positive effects were observed on seed priming with 2% jamun (*Syzygium cumini*) leaf extract or water (hydro-priming), which significantly accelerated total amylase and alcohol dehydrogenase activities in rice (Ella et al., 2011; Sarkar, 2012).

Moreover, this research examined the influence of traditional and advanced methods of priming on chlorophyll content. As shown, statistical analysis (Figures 4.13 and 4.15) demonstrated significant variability among seeds treated under the 45-day storage condition. Especially priming time has affected chlorophyll a, and chlorophyll total (Chl-T) content led to an increase in plants that treated their seeds for 9 hours in the solutions, also nano-primed plants at 10 ppm concentrations of TiO₂ nanoparticles increased significantly. In contrast, seeds treated with TiO₂ NPs showed the opposite effect within 3 hours related to chlorophyll a. while Chlorophyll a level in all treated plants that were grown under the 10-day storage condition revealed a similar pattern in comparison to the control group. The study also found a comparable outcome in marjoram (*Origanum majorana* L.) leaves, where priming seeds with nanoparticle TiO₂ at concentrations of 20 and 40 mg L⁻¹ resulted in elevated levels of chlorophyll in the leaves (Jafari et al., 2022). Safaeipour et al., (2023) noted a significant increase in stevia of chlorophyll content, for seed primed using various nano-compounds (zinc oxide, titanium oxide, or silicon) under the PEG-6000 condition at different intensities (0, 0.3, -0.6, and -0.9 MPa). Samadi et al., (2014) showed that the application of TiO₂ NPs (200 mg/L) on *Mentha* sp. resulted in a favorable impact on chlorophyll a, chlorophyll b, and carotenoid contents.

Typically, seed priming helps to boost the number of mitochondria and enhance the integrity of the mitochondrial outer membrane as well as pigments of

photosynthetic, especially chlorophyll content that indicates potential influence on photosynthesis function in plants. Notably, they provoke a photosystem complex structure that leads to the transport, absorption, and trapping of electrons per photosystem II (Benamar et al., 2003; Jisha & Puthur, 2016; Varier et al., 2010).

In standard growth environments, plants maintain a fluctuating balance between generating and removing reactive oxygen species (ROS). However, exposure to various abiotic stresses can disrupt this balance, leading to increased ROS generation and accumulation (Ashraf & Harris, 2004; P. Das et al., 2015). ROS, including superoxide radicals and hydrogen peroxide, have the potential to inflict harm on cellular macromolecules like proteins, nucleic acids, and lipids, thereby impacting cellular structure and the antioxidative system (Ashraf & Foolad, 2005; McDonald, 1999; Munns & Tester, 2008).

Lipid peroxidation, particularly the peroxidation of polyunsaturated fatty acids, is identified as a crucial molecular mechanism inhibiting seed germination under abiotic stress conditions (Yang et al., 2010). The generation of ROS induced by abiotic stress enhance the levels of malondialdehyde (MDA), a highly mutagenic byproduct of lipid peroxidation, which can induce damage to cellular macromolecules (Ali et al., 2017; Xu et al., 2015). The content of MDA serves as an indicator of oxidative damage at the cellular membrane level under abiotic stress, indicating the extent of harm inflicted on plant tissues (Gong et al., 2008; Weisany et al., 2012).

Moreover, seed priming diminishes the build-up of malondialdehyde (MDA), which rises in response to abiotic stresses and serves as an indicator of oxidative stress. This decline in MDA levels is linked to enhanced membrane repair and the triggering of antioxidant enzyme responses during the seed priming process (Dong et al., 2014; Paparella et al., 2015).

This research essayed MDA as a crucial stress-induced biomarker that shows significant cellular injury due to scanty ability to insufficient the excess ROS production. As shown statistical analysis in Figure 4.16 did not exhibit significant changes in MDA content in SG188 sugar beet seedlings ($p < 0.05$). Across all treatments, the MDA content of seedlings that their seeds were stored about 10 days after priming was slightly lower than seed primed storage for 45 days. In a different study, the nano-priming of maize with TiO_2 at a concentration of 60 ppm, under salt stress conditions, resulted in heightened activities of superoxide dismutase

(SOD) and catalase (CAT), along with a decrease in malondialdehyde (MDA) content and membrane electrolyte leakage (MEL) when compared to the control group (Shah et al., 2021b). Priming mung beans (*Vigna radiate*) with various concentrations of β -Amino butyric acid (0, 0.5, 1.0, 1.5, 2.0, and 2.5 mM) for 6 hours resulted in reduced malondialdehyde (MDA) levels (Jisha & Puthur, 2016). Moreover, in wheat (*Triticum sativum*) primed with either dH₂O or CaCl₂/1.5% for 12 hours (Solution ratio 1:5, w/v) exhibited decreased MDA and sodium content (Tabassum et al., 2017). Priming stressed crops with zinc nanoparticles in lupine plants led to a reduction in MDA and Na⁺ content (Abdel Latef et al., 2017). In addition, the hydro-priming of alfalfa seeds for 12 hours at 25 $\pm$ 1°C under high level of salt content (150 mM NaCl) stress conditions decreased malondialdehyde (MDA) accumulation and electrolyte leakage (Amooaghaie, 2011). Another finding of the hydro-priming technique showed that soaking Napa cabbage (*Brassica rapa* subsp. *pekinensis*) seeds in water for 10 hours under salt stress conditions improved both seed germination percentages and early seedling growth. These positive effects were strongly associated with increased activities of peroxidase (POD) and catalase (CAT), along with proline accumulation, which aids in mitigating oxidative membrane damage, consequently leading to reduced concentrations of MDA (M. Yan, 2016). When the data in the study were examined for 10 days and 45 days post priming, it was observed that there was a limited increase in the MDA activity of all primed seeds compared to the control, and this increase tends to increase according to the application time. However, MDA activity in seeds exposed to only 15 ppm TiO₂ for 9 hours decreased compared to 3 and 6 hours.

6. CONCLUSIONS AND RECOMMENDATIONS

This study focused on the sugar beet of SG188 genotype. It demonstrated an ineffective influence on germination parameters in both hydro-priming and nano-priming techniques with titanium dioxide nanoparticles (5,10,15 ppm) as solution priming under the 3,6, 9 hours priming time and 10, 45 days of post-priming maintenance seeds. Although traditional and advanced methods showed different influences on physiological and biochemical parameters, they did not exhibit adverse effects on the germination percentage of sugar beet seedlings. Generally, hydro-priming displayed a germination rate and vigor increase in comparison with the control group. Moreover, alpha-amylase as enzymatic activity, chlorophyll a, and chlorophyll total contents demonstrated a significant statically increase that led to enhanced metabolic activation and positive consequences on photosynthetic efficiency during germination of seeds. For the sugar beet of SG188 genotype, it was determined that the nano-primed seeds with titanium dioxide nanoparticle at 10 ppm concentration treatment exhibited the best results in seedling vigor and seed germination rate, and nano-primed seeds increased alpha-amylase activity enzyme, although slightly less than hydro-priming. In addition, a substantial rise in chlorophyll content was revealed in seedlings that were primed with TiO₂ nanoparticles at 10 ppm concentration. Across all nano-primed seeds that were soaked in priming solution for 9 hours, the level of MDA led to an increase due to oxidative stress that indicate ROS make potential damage. In conclusion, both hydro-priming and nano-priming positively affected seedlings of the sugar beet SG188 genotype. Hydro-priming effect on enzymatic activity and chlorophyll level while advanced method with titanium dioxide nanoparticles had significant benefits in reducing oxidative stress and boosting seedling vigor in 3 hours priming time. The finding of this research involved seed pre-germinative metabolism, and early seedling growth that suggests valuable insights for optimizing seed treatments to improve crop performance under diverse environmental conditions in agriculture.

7. REFERENCES

APA citation system was used in this thesis.

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